Mellon,[6] who believed its apparent absence was due to temporary suppression of a special "growth phase" possessing this function. Whether suppression of peroxide formation in the white colonies is complete, or reduced to a degree not detectable by the present methods, remains a question.

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Microdetermination of Homocystine in Pure Solution with the Dropping Mercury Cathode.*

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In a previous paper the polarographic microdetermination of cystine in globin hydrolysates was reported.[1] Under the conditions outlined for the experiment it was shown that methionine did not give a polarographic effect and was not detected by the mercury cathode. The fact that methionine does not interfere in the polarographic determination of cystine, provided its concentration is not greatly in excess of that of cystine, has been verified by Smith and Rodden.[2] A special calibration method was devised, however, to eliminate any errors in the cystine determination should interference arise from methionine or other amino acids present in the hydrolysates.[1]

Homocystine, which is derived from methionine by demethylation and oxidation, gives the sulfhydryl compound, homocysteine, upon reduction. Inasmuch as homocystine is a homologue of cystine, the behavior of these compounds should be analogous. While homocystine may be determined by the method of Okuda[3, 4] and photometrically,[5] the physiological importance of homocystine makes it desirable to have still another method for determining this substance

[6] Mellon, R. R., 1940, in press.

* A preliminary report of this paper was presented before the Division of Biological Chemistry at the Ninety-seventh Annual Meeting of the American Chemical Society at Baltimore, Md., April 3-7, 1939.

[1] Stern, A., Beach, E. F., and Macy, Icie G., *J. Biol. Chem.*, 1939, **130**, 733.

[2] Smith, E. R., and Rodden, C. J., *J. Research Nat. Bur. Standards*, 1939, **22**, 669.

[3] Okuda, Y., *J. Biol. Chem.* (Japan), 1925, **5**, 17.

[4] Brand, E., Cahill, G. F., and Block, R. J., *J. Biol. Chem.*, 1935, **110**, 399.

[5] Kassell, Beatrice, *J. Biol. Chem.*, 1935, **109**, xlix.

in low concentrations. For this reason the possible catalytic effect of homocystine at the dropping mercury cathode in the presence of ammonia, ammonium chloride and cobaltous chloride has been investigated in the hope that it might furnish the basis of a method for determining homocystine in solutions.

The homocystine used in these experiments was prepared by the method of Butz and Du Vigneaud.[6] The determinations were carried out with a standard solution of 10^{-2} M homocystine in 0.177 N hydrochloric acid. Shortly before making the measurements, 1 cc of this standard solution was added to a mixture of ammonium chloride and ammonia in a 25 cc volumetric flask and diluted so that the final concentration of the diluent was 0.1 N ammonium chloride and 0.1 N ammonia. Successively, different amounts of the standard solution of homocystine were introduced into 25 cc volumetric flasks and diluted to the mark with varying amounts of stock solutions of ammonium chloride, ammonia and cobaltous chloride to make the final concentration always the same (0.1 N ammonia, 0.1 N ammonium chloride and 10^{-2} M cobaltous chloride) in accordance with the specifications of Brdicka[7] for cystine. The polarograms for several different concentrations of homocystine were measured with the Heyrovsky polarograph. (Oxygen need not be removed from the solutions.)

It was found in the present work, as Brdicka[7] determined with several other thio-acids, that homocystine gives the polarographic effect in the presence of ammonia, ammonium chloride and cobaltous chloride. It is seen in Fig. 1 that the catalytic wave of the current voltage curve of homocystine does not exhibit the rounded maximum characteristic of cystine.[1] In this respect the homocystine current voltage curve resembles that of glutathione and of metallic ions in the presence of colloidal substances. To eliminate the possibility that impurities might cause the catalytic effect the compound was recrystallized several times from water. After many recrystallizations the homocystine still gave a characteristic catalytic wave without a rounded maximum and equimolar solutions of the compound before and after recrystallization showed the same wave heights. Homocystine, like cystine, does not give the effect except in the presence of cobaltous ions.

A calibration curve for homocystine in pure solution may be constructed by plotting wave heights against concentration, in the usual

[6] Butz, L. W., and Du Vigneaud, V., *J. Biol. Chem.*, 1932-33, **99**, 135.

[7] Brdicka, R., *Collect. Czechoslov. Chem. Communicat.*, 1933, **5**, 148.

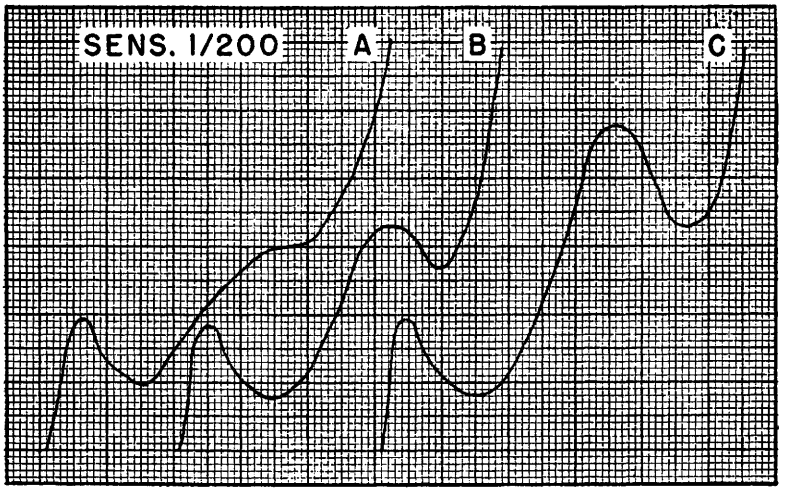

FIG. 1.
Typical current voltage curves in solutions with concentration of 0.1 *N* NH_4Cl, 0.1 *N* NH_3, and 10^{-2} *M* $COCl_2$. Curve A, homocystine. Curve B, mixture of homocystine and cystine. Curve C, cystine.

manner. By means of such a curve homocystine may be quantitatively determined in pure solutions in concentrations of only 26 γ per cc with an accuracy of ±3%. The increases in wave heights of current voltage curves of homocystine, as with cystine, are not directly proportional to the concentration.

The wave height of a solution of homocystine is somewhat lower than that of an equimolar solution of cystine. The wave heights seem to vary with the structure of the thio acids. Brdicka[7] has studied the relation of wave height to the proximity of the sulfhydryl group to the amino acid group. The wave heights of equimolar solutions of the substances studied decrease in the order thioglycolic acid, cysteyl-glycine, cysteine. The wave heights of homocysteine are lower than that of cysteine. This indicates that there is a relation between the wave heights and the proximity of the amino to the sulfhydryl group. Otherwise it is not clear why the wave height of cysteyl-glycine is higher than that of cysteine. The relations between wave height and structure, however, seem to be more complicated since the character of the thiol group is also influenced by its proximity to the carboxyl group as well as to the amino group.

Homocysteine, like homocystine, has also been found to give the catalytic wave without the rounded maximum at the dropping mercury cathode. The mechanism of the polarographic reaction of homocystine no doubt is similar to that of its lower homologue, cystine;

the homocystine is reduced to 2 molecules of homocysteine before the potential of the catalytic wave is reached. It is then homocysteine which reacts with the cobaltous ions. It is of interest that with the dropping mercury cathode homocystine is readily reduced, whereas the catalytic hydrogenation of Bergmann and Michaelis[8] which is successful when applied to cystine fails to reduce homocystine.[4] Since the polarographic effect is due to the sulfhydryl groups, the potential at which the cathode reaction takes place is practically the same for homocystine and homocysteine as it is for other thio acids such as cystine and cysteine. Therefore, it is thus far impossible to distinguish qualitatively between homocystine and cystine by the polarographic method if they are in solution together. However, in instances in which the absence of glutathione is certain, a current voltage curve without rounded maximum would be suggestive of the presence of homocystine alone.

On the other hand, an investigation of the current voltage curves given by mixtures of cystine and homocystine shows that it is possible to determine both amino acids quantitatively in the same solution. In this case the curves exhibited the rounded maximum characteristic of cystine and other thio acids (Fig. 1). Furthermore, the sum of the quantities of homocystine and cystine together, in the relative proportions thus far studied, can be calculated from the calibration curve for cystine alone and with the same accuracy. This means that the wave height due to homocystine in solution with cystine is augmented sufficiently to be almost the same as that due to an equivalent quantity of cystine sulfhydryl groups. This observation is of significance when it is recalled that homocystine in the Folin and Marenzi method[9] develops considerably less color than an equimolar amount of cystine whereas together in solution cystine and homocystine produce the full amount of color to be expected if all of the sulfhydryl groups were in the form of cystine.[4]

The polarographic method furnishes a means of determining homocystine which may be applied to biological material. Obviously it would be of value in determining methionine after quantitative demethylation. Investigations of these problems and particularly those relating to the determination of methionine are now in progress.

Summary. Homocystine and homocysteine can be determined quantitatively with the polarographic method. In concentrations as low as 26 γ per cc the accuracy is ±3%. The quantity of sulfhydryl

[8] Bergmann, M., and Michaelis, G., *Ber. deutsch. chem. Ges.*, 1930, **63B**, 987, cited, *Chem. Abs.*, 1930, **24**, 3757.

[9] Folin, O., and Marenzi, A. D., *J. Biol. Chem.*, 1929, **83**, 103.

groups in mixtures of homocysteine and cystine can be determined with the same accuracy although it is not possible to distinguish homocystine and cystine qualitatively by this method.

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Conversion of S-benzylglutathione to Benzylmercapturic Acid in the Rat.*

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We reported recently that benzyl chloride, when administered to dogs, rabbits, and rats, yields in the urine of these animals N-acetyl-S-benzylcysteine. The same mercapturic acid was obtained from the urine of dogs, rabbits, and rats on feeding S-benzylcysteine to the animals.[1]

When S-benzylhomocysteine was administered to rabbits and rats N-acetyl-S-benzylhomocysteine was excreted in the urine.[2] This finding indicated that homocysteine is not the intermediate substance in the synthesis of benzylmercapturic acid from benzyl chloride *in vivo* and that benzylhomocysteine is not convertible to benzylcysteine in the animal body. The acetylation of S-benzyl-cysteine and S-benzylhomocysteine in the rat was confirmed.[3]

The study of the metabolism of the benzyl derivatives of sulfur-containing amino acids and their derivatives has now been extended to S-benzylglutathione. The experiments described here deal with the fate of S-benzylglutathione in the rat.

Experimental. Synthesis of S-benzylglutathione. 1.0 g of commercial glutathione was dissolved in about 30 cc of liquid ammonia and dry metallic sodium was added to the solution in small portions until a permanent blue coloration was obtained. An excess of benzyl chloride (0.5 cc) was then added dropwise, with shaking, and the ammonia was allowed to evaporate. The residue was then extracted with ether 3 times and then dissolved in water. Enough concentrated HCl was added to the solution to obtain a pH of 4 to 4.5, and

* Grateful acknowledgment is made to Mr. J. Alicino for the microanalytical work.

1 Stekol, J. A., *J. Biol. Chem.*, 1938, **124**, 129.

2 Stekol, J. A., *J. Biol. Chem.*, 1939, **128**, 199.

3 du Vigneaud, V., Wood, J. L., and Irish, O. J., *J. Biol. Chem.*, 1939, **129**, 171.