9030 P

Carboxymethylcysteine Metabolism, its Implications on Therapy in Cystinuria and on the Methionine-Cysteine Relationship.

ERWIN BRAND, RICHARD J. BLOCK, BEATRICE KASSELL AND GEORGE F. CAHILL.

From the Department of Chemistry, N. Y. State Psychiatric Institute and Hospital and from the Squier Urological Clinic of the Presbyterian Hospital, New York.

In continuation of our experiments on sulfur metabolism, the behavior of carboxy-methyl-S-cysteine[1] (I) was investigated. (We are indebted to Dr. L. Michaelis for suggesting this compound for study.) Carboxyl-methyl-S-cysteine, like methyl-S-cysteine[2] does not support the growth of rats on a sulfur deficient diet, indicating that (I) does not yield cysteine (IV) in the course of its metabolism.

Three and six-tenths grams of (I) were administered to a normal human being, while 7.2 and 14.4 gm. respectively were given to 2 cystinurics. The substance was not toxic and yielded in the urine neither cystine nor –SH compounds. In the normal, the sulfur of (I) was only partially oxidized (40%), the larger portion (60%) being excreted as undetermined neutral S. Part of this neutral S was apparently a disulfide, since there appeared in the urine a strong cyanide-nitroprusside reaction, while the Sullivan test remained negative. The urine was discarded before the probable nature of this disulfide was realized.

Following the ingestion of (I) by the cystinuric patients, about 15% of the extra sulfur excreted was inorganic sulfate and 85% undetermined neutral S. Cystine excretion remained practically unchanged as indicated by the Folin photometric method,[3] but when measured by the Sullivan and Lugg-Sullivan methods, it dropped from one gm. to 200 mg. per day.

These various findings seemed to indicate that part of (I) was excreted presumably as the unchanged compound, that part of it was oxidized to yield inorganic sulfate, and that another portion was excreted as an intermediate which contained sulfur in the form of a disulfide linkage. As a result of previous experiments,[4] and

[1] Michaelis, L., and Schubert, M. P., *J. Biol. Chem.*, 1934, **106**, 331.

[2] Block, R. J., and Jackson, R. W., *J. Biol. Chem.*, 1932, **97**, cvi; duVigneaud, V., Loring, H. S., and Craft, H. A., *J. Biol. Chem.*, 1934, **105**, 481.

[3] Kassell, B., *J. Biol. Chem.*, 1935, **109**, xlix.

[4] Brand, E., Cahill, G. F., and Harris, M. M., *J. Biol. Chem.*, 1935, **109**, 69; Brand, E., Cahill, G. F., and Block, R. J., *J. Biol. Chem.*, 1935, **110**, 399.

from the chemical formula of (I), it was reasonable to assume that the excretion of a disulfide resulted from the oxidation of an –SH compound derived from carboxymethylcysteine. The observations could therefore be explained by assuming that in the catabolism of (I), thioglycolic acid (II) is formed, which is excreted as dithiodiglycolic acid (III).

$$\underset{\text{Carboxymethylcysteine}}{\overset{\text{I}}{HOOC \cdot CH_2—S—CH_2 \cdot CH(NH_2) \cdot COOH}} \rightarrow \underset{\text{Thioglycolic acid}}{\overset{\text{II}}{HOOC \cdot CH_2 \cdot SH}} \rightarrow \underset{\text{Dithiodiglycolic acid}}{\overset{\text{III}}{(—SCH_2 \cdot COOH)_2}}$$

This conception seemed to be substantiated by the finding that both (II) and (III) markedly depress color formation in the Sullivan and Lugg-Sullivan reactions. Although (II) and (III) are readily soluble in ether, extraction of the cystinuric urines from the experimental periods did not yield any ether-soluble S-S compounds.

Our experiments[4] have indicated that the metabolic error in cystinuria is concerned with the handling of cysteine and that there are probably present in the kidney of the cystinuric individual abnormally large amounts of cysteine. It was therefore conceivable that in the presence of large amounts of cysteine (IV) the thioglycolic acid (II) resulting from the catabolism of (I) was excreted as a mixed disulfide such as (V).

$$\underset{\text{Thioglycolic acid}}{\overset{\text{II}}{HOOC \cdot CH_2 \cdot SH}} + \underset{\text{Cysteine}}{\overset{\text{IV}}{HS \cdot CH_2 \cdot CH(NH_2) \cdot COOH}} \rightarrow \underset{\text{Mixed disulfide}}{\overset{\text{V}}{HOOC \cdot CH_2 \cdot S—S \cdot CH_2 \cdot CH(NH_2) \cdot COOH}}$$

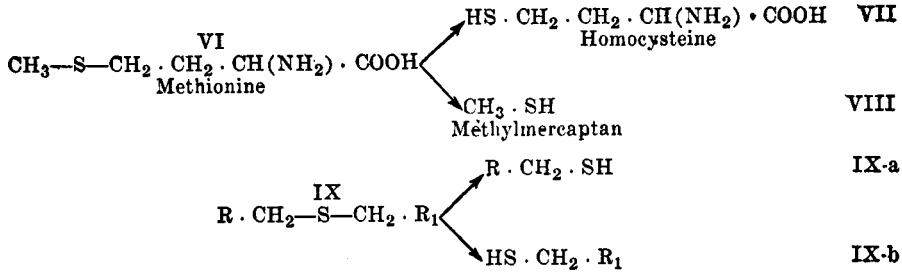
Although a compound such as (V) has as yet not been isolated, its presence is indicated by the following observations. On reduction (Zn + HCl) of 25-100 cc. of the experimental urine it was possible to detect the characteristic odor of thioglycolic acid. Ether extracts of the reduced urine contained a substance with acidic properties, which gave a strong nitroprusside reaction, Goddard and Michaelis's[5] test for thioglycolic acid and a reaction curve in the Folin-photometric determination similar to that given by thioglycolic acid.

Mixed disulfides are quite soluble. The probable formation of a compound such as (V) following the administration of (I) to cystinuric patients may, therefore, have therapeutical implications regarding the formation of cystine stones. In this connection it is interesting to note that during the period of feeding of (I) cystine crystals disappeared from the urine of one of the cystinuric patients.

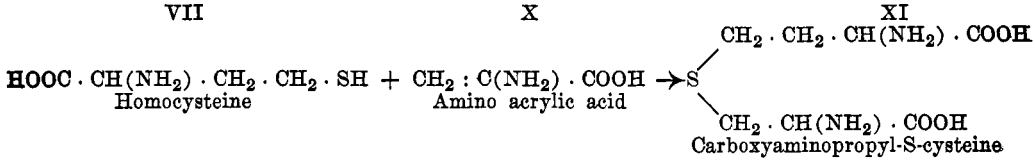
Since methionine (VI) can be split[7] to yield homocysteine (VII)

[5] Goddard, D. R., and Michaelis, L., *J. Biol. Chem.*, 1934, **106**, 605.

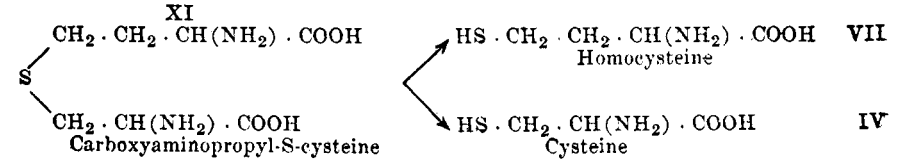
or methylmercaptan (VIII) and carboxymethylcysteine to yield thioglycolic acid (II), it appears that compounds of the general formula (IX) may be split to yield (IX-a) or (IX-b), depending upon conditions and the nature of the substituents R and R_1.



It has been stated[4] that, along the line suggested by model experiments,[6] the conversion of homocysteine (VII) into cysteine (IV) may be accomplished by a reaction of (VII) with amino acrylic acid (X) (or with its peptides) to yield carboxyaminopropyl-S-cysteine (XI) (or its peptides).



It is therefore conceivable that (XI) may be split to yield cysteine (IV).



These conceptions form the basis for a working hypothesis regarding some of the mechanisms by which the conversion of methionine into cysteine may be accomplished in intermediary metabolism, and also indicate the importance of synthesizing (XI) and its derivatives for chemical and metabolic investigations. (XI) has on one occasion been isolated from wool by Kuester and Irion.[8]

Note. Recently, 14.4 gm. of (I) were administered to another human being. The urine of the experimental period contained approximately 800 mg. of the mixed disulfide (V).

[6] Nicolet, B. H., *Science*, 1935, **81**, 181; *J. Biol. Chem.*, 1932, **95**, 389; Bergmann, M., *Naturwissinschaften*, 1934, **22**, 135.

[7] Butz, L. W., and duVigneaud, V., *J. Biol. Chem.*, 1932-33, **99**, 135; *cf.* Virtue, R. W., and Lewis, H. B., *J. Biol. Chem.*, 1934, **104**, 59.

[8] Kuester, W., and Irion, W., *Z. physiol. Chem.*, 1929, **184**, 225.