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Effect of pyridoxine treatment of a homocystinuric patient on the urinary excretion of some sulfur-containing amino acids

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EFFECT OF PYRIDOXINE TREATMENT OF A HOMOCYSTINURIC PATIENT ON THE URINARY EXCRETION OF SOME SULFUR-CONTAINING AMINO ACIDS

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Abstract: The effect of pyridoxine treatment of a homocystinuric patient on the urinary excretion of some sulfur-containing amino acids was studied and the following results were obtained. As a result of pyridoxine treatment, urinary homocystine decreased to a fairly great extent, and its unusual metabolites S-(3-hydroxy-3-carboxy-n-propylthio) homocysteine (HCPTHC) and S-(β-carboxyethylthio) homocysteine (β-CETHC) increased to some extent. But its oxidation product (homocysteic acid) showed a tendency to decrease slightly. Urinary methionine and cystine increased to some extent, but cysteine-homocysteine mixed disulfide showed no remarkable change.

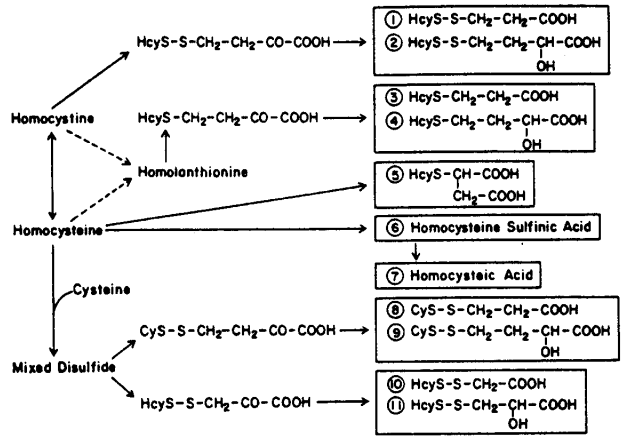
At least two types of homocystinuria have been recognized: one is pyridoxine-sensitive and the other fails to respond to pyridoxine treatment, and most of these investigations are concerned with the changes of methionine, homocystine and cystine in blood and urine. Recently, however, many unusual sulfur-containing amino acids shown in Fig. 1 have been found in the urine of homocystinuric patient (1–4), so the studies of pyridoxine effect on the urinary excretion of these unusual metabolites will be of great interest. Chemical terms and abbreviations of unusual sulfur-containing amino acids are listed in Fig. 1.

MATERIALS AND METHODS

Pyridoxine hydrochloride (200 mg/dl) was administered daily to a homocystinuric patient found in Osaka for two weeks from October 12 to 26 in 1972, and four urine samples were collected as follows: 1. Control urine before B_6 treatment (6.2 liters), August 24-31; 2. B_6 Administration I in Table 1 (2.6 liters), October 13-19; 3. B_6 Admimstration II (3.8 liters), October 20-26; 4. After B_6 Administration (3.9 liters), November 6-12.

According to our usual method (5), two liters each of urine samples was at first divided into two fractions by using a column containing one liter of Diaion SK-1 (H form, mesh 100), and so-called Fraction I (5) was further divided into

Fig. 1. Unusual Metabolism of Homocysteine in Homocystinuric Patents.



		Abbreviations
1.	S-(β-carboxyethylthio) homocysteine	β-CETHC
2.	S-(3-hydroxy-3-carboxy-n-propylthio) homocysteine	HCPTHC
3.	S-(β-carboxyethyl) homocysteine	β-CEHC
4.	S-(3-hydroxy-3-carboxy-n-propyl) homocysteine	HCPHC
5.	S-(1, 2-dicarboxyethyl) homocysteine	DCEHC
8.	S-(β-carboxyethylthio) cysteine	β-CETC
9.	S-(3-hydroxy-3-carboxy-n-propylthio) cysteine	HCPTC
10.	S-(carboxymethylthio) homocysteine	CMTHC
11.	S-(2-hydroxy-2-carboxyethylthio) homocysteine	HCETHC

TABLE 1 EFFECT OF PYRIDOXINE ADMINISTRATION ON THE EXCRETION OF SULFUR-CONTAINING AMINO ACIDS IN THE URINE OF A HOMOCYSTINURIC PATIENT

	Before	B6 Administration I	B6 Administration II	After
Methionine	82.0	129.0	210.0	490.0
Homocystine	260.0	110.0	131.0	157.0
Mixed disulfide	337.0	233.0	345.8	281.8
Cystine (Half)	49.3	44.0	143.0	89.0
Homocysteic acid	6.72	6.21	4.86	2.20
β-CETHC	0.30	0.95	1.53	0.74
HCPTHC	5.17	22.10	15.40	26.50

Values are expressed in μ moles/liter

three fractions by using a column containing 400 ml of Amberlite IRA-68 (acetate form, mesh 50). Fractions Ib and Ic thus obtained were combined and transferred to a column containing 500 ml of Diaion SK-1 (H form, mesh 100). The effluent and water washings were evaporated to dryness and used for the determination of homocysteic acid.

After washing with water, the column was further washed with 2000 ml of 2 N HCl, 2000 ml of water, and then eluted with 1000 ml of 2 N NH_3. The ammonia eluate containing β-CETHC and HCPTHC was evaporated to dryness and analyzed on an automatic amino acid analyzer (Hitachi Model 034 Liquid Chromatograph). Cystine, methionine, mixed disulfide and homocystine were analyzed directly on an amino acid analyzer after hydrolysis of each original urine without further fractionation.

RESULTS AND DISCUSSION

Results of pyridoxine effect on the urinary excretion of several sulfur-containing amino acids are summarized in Table 1.

The excretion of homocystine tends to decrease after pyridoxine treatment and its unusual metabolites (β-CETHC and HCPTHC) increase to some extent. It is well known that cysteine and also homocysteine react with pyridoxal to give thiazolidine derivatives and the latter derivatives cannot participate directly in pyridoxal-catalyzed reactions (6). So the reduced excretion of homocystine after B_6 treatment might be attributed to some extent to the formation of thiazolidine derivative. But it cannot be denied that pyridoxine treatment enhances the deamination of homocysteine or homocystine, because of the excretion of its degradation products (β-CETHC and HCPTHC) being increased.

Decreasing tendency of urinary homocysteic acid may be due to the reduction of homocysteine in tissues. Even if the amount of mixed disulfide is taken into account, the amount of urinary homocystine still has a tendency to decrease and that of cystine to increase. As described above, cysteine also reacts with pyridoxal to give a thiazolidine derivative. So the increasing tendency of cystine excretion after B_6 treatment is inexplicable at present. Even if the cystathionine synthase remaining in tissues of the patient is considered to be activated by B_6 treatment, it is still incomprehensible why urinary methionine tends to increase after B_6 treatment. At any rate, such a patient has not been encountered as yet.

The other sulfur-containing amino acids listed in Table 1 could not be measured at this time, because of the repeated fractionations being required for their determinations as reported in previous papers (1–4).

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