Cystathioninuria from Pyridoxine Deficiency Complicating Treatment of Hypercalcaemia in a Cretin

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Rats with vitamin B6 deficiency excrete cystathionine in the urine (Hope, 1957). Cystathionine is an amino acid in the pathway of synthesis of cysteine from methionine (Fig. 1). A methyl group is removed from methionine to produce homocysteine, which condenses with serine to yield cystathionine. This condensation is catalysed by a transulphurase enzyme requiring pyridoxal phosphate as a co-enzyme. Finally, cystathionine splits to yield cysteine and homoserine. This reaction is catalysed by cystathionase and also requires pyridoxal phosphate (White, Handler, and Smith, 1964). Pyridoxal and its phosphate are forms of vitamin B6, the others being pyridoxamine and its phosphate, and pyridoxine. Rats with vitamin B6

deficiency may continue to make cystathionine but they fail to split it.

Pyridoxal phosphate is a co-enzyme for many other enzymes that are important in metabolism (Holtz and Palm, 1964), including certain transaminases and decarboxylases. Several of the enzymes concerned in tryptophan metabolism require pyridoxal phosphate. The net effect of vitamin B6 deficiency on tryptophan metabolism is usually to increase the excretion of xanthurenic acid, particularly after the addition of a dose of tryptophan to the diet, but the precise reason for the increased xanthurenic acid excretion is not clear (Greenberg, Bohr, McGrath, and Rinehart, 1949; Holtz and Palm, 1964). The increase has served to demonstrate a deficiency of vitamin B6 (Bessey, Adam, and Hansen, 1957), though it does not always accompany

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FIG. 1.—The synthesis and degradation of cystathionine.

a deficiency. Whether or not there is an increase in xanthurenic acid excretion may depend on how the deficiency was induced and on the species of the animal. Scriver and Hutchison (1963) have also found that the excretion of taurine may be reduced.

Snyderman, Holt, Carretero, and Jacobs (1953) deprived 2 infants of pyridoxine. One, aged 2 months, stopped growing after 33 days, and after 76 days had convulsions that were controlled by a single dose of 50 mg. pyridoxine. The other, aged 8 months, stopped growing after 73 days; his Hb concentration began to fall after 30 days. The anaemia responded to 50 mg. pyridoxine. The infants were unable to convert tryptophan to N¹-methylnicotinamide. The excretions of xanthurenic acid and of cystathionine were not measured.

Pyridoxine deficiency appeared in many infants given 'Synthetic Milk Adapted' (SMA, Wyeth) before the need to add pyridoxine to this preparation was recognized (May, 1954). Three out of every 1,000 infants given the preparation had fits after 1 to 4 months (Bessey *et al.*, 1957), and they had an abnormal excretion of xanthurenic acid after tryptophan. Cystathionine was not looked for. The abnormalities were corrected with 0.2 - 0.4 mg. pyridoxine daily.

Some breast-fed infants have the clinical and biochemical abnormalities of pyridoxine deficiency. Bessey *et al.* (1957) described two with convulsions and an abnormal excretion of xanthurenic acid. This persisted until they received 2 mg. daily of vitamin B₆. Scriver and Hutchison (1963) also described an infant aged 3 months with convulsions who had an abnormal excretion of xanthurenic acid. This infant had cystathioninuria. All the abnormalities were abolished by 2.3 - 2.75 mg. pyridoxine daily. The infants of Bessey *et al.* and of Scriver and Hutchison differed from those with pyridoxine deficiency after SMA, in that their requirement for pyridoxine was about 4 times the normal. But their intestinal absorption and renal excretion of the vitamin were normal.

There is another group of infants with convulsions responding to pyridoxine in doses varying from 2 to 15 mg. daily, who excrete normal amounts of xanthurenic acid (Hunt, Stokes, McCrory, and Stroud, 1954). One, at least, of these infants also had normal amounts of cystathionine in the urine (Scriver and Hutchison, 1963). It has been suggested that in these infants the formation of γ -amino-butyric acid in the brain requires larger than normal amounts of B₆, but that there is no general abnormality of B₆ metabolism (Scriver, 1960). The term B₆-dependency has been invented

to describe these cases, the term vitamin B₆-deficiency being reserved for those with the other abnormalities, such as arrest of growth and abnormal metabolism of tryptophan, characteristic of B₆ deficiency.

Hagberg, Hamfelt, and Hansson (1964) have found that fits associated with a disturbed metabolism of tryptophan and responding to treatment with pyridoxine happen not only in infants but in older children as well. They described 3 children with fits and progressive mental disturbances in whom 60-160 mg. pyridoxine arrested the fits and the mental deterioration.

Cystathioninuria has only once been described in an infant (Scriver and Hutchison, 1963). It has been described in adults. The cystathioninuria found in one patient with a neuroblastoma and one with an argentaffinoma (Gjessing, 1963) probably reflected the metabolism of the tumours rather than any deficiency of pyridoxine. In a survey of patients in a mental hospital, Harris, Penrose, and Thomas (1959) found one woman aged 64 with abnormally large amounts of cystathionine in the urine. They did not try the effect of pyridoxine. Frimpter, Haymovitz, and Horwith (1963) also described an adult with cystathioninuria. He was not mentally defective; he had an abnormal excretion of xanthurenic acid after tryptophan. The biochemical abnormalities could be corrected with the large dose of 90 mg. daily of pyridoxine.

We describe cystathioninuria from simple pyridoxine deficiency. The patient was a cretin whose treatment with thyroxine was complicated by hypercalcaemia (Royer, Lestrade, and Habib, 1958). Cystathioninuria appeared when the hypercalcaemia was treated with a purified low-calcium diet.

Case Report

D.D. was born on March 8, 1963, 3 weeks after the expected date of delivery. She weighed 3.1 kg. at birth. She was the fourth child of healthy parents; her 3 sisters were well. She was admitted altogether 3 times. Her first admission was from May 27, 1963, to July 15, 1963, for the investigation and treatment of cretinism. Her second admission was from August 30, 1963, to February 20, 1964, for hypercalcaemia. It was during the treatment of the hypercalcaemia with a low calcium preparation of milk that she was found to have cystathionine in the urine. Her third admission was from September 1, to September 10, 1964, for review of the diagnosis of cretinism.

Cretinism. When she was first admitted at the age of 11 weeks she moved little and cried rarely; her cry was hoarse. She was unresponsive and always had to be roused from sleep to be fed. Her features were coarse; her skin was thick, scaly, and yellow; her hair was scanty

TABLE I
Effect of Thyroid Extract on PBI, Serum Cholesterol, and Appearance of Epiphyses in Wrist

Date*	Cholesterol (mg./100 ml.)	PBI (μ g./100 ml.)	Ossification Centres in Wrists	Thyroid Extract (mg./day)
June 6, 1963	—	1.5	—	—
June 14, 1963	225	—	—	—
July 14, 1963	—	—	—	48
September 20, 1963	—	8.5	0	64
October 29, 1963	190	—	0	64
January 22, 1964	120	—	—	64
February 19, 1964	139	—	—	64
May 20, 1964	227	1.3	1	64†
July 1, 1964	292	1.2	1	—
September 2, 1964	400	1.7	1	—

* Date of birth March 8, 1963. † Treatment stopped, resumed September 7, 1964, with L-thyroxine.

and fine. Her abdomen was distended, her colon and rectum were full, and she had an umbilical hernia. Her muscle tone was diminished. Her weight was 4.2 kg., her length 55 cm. Her temperature ranged from 96° to 99° F. (35.6-37.2° C.). She was obviously a cretin. This was confirmed by a low concentration of protein-bound iodine (PBI) in the plasma, 1.5 μ g./100 ml. (normal 4 to 8 μ g./100 ml.). Furthermore, the phosphocreatine kinase activity in the serum was raised, 15 units/100 ml. (normal less than 4 units/100 ml.). The ossification centres in the knee had not appeared.

Treatment was started with thyroid extract 8 mg. daily on June 17 when she was 14 weeks old. This dose was increased to 48 mg. daily by July 14, 1963, when she was discharged from hospital, and further to 64 mg. daily on September 20 (Table I).

Hypercalcaemia. She was readmitted after she had been treated for 9 weeks because she was still constipated and she lacked any interest in her feeds, though she was alert and happy and had lost her cretinous appearance. Her weight had increased only to 4.4 kg. She now had an aortic systolic murmur; her blood pressure was 100/70 mm. Hg. These symptoms and signs, and her facies described as 'elfin' suggested the diagnosis of hypercalcaemia. Her serum calcium was 13.9 mg./100 ml. (Zeiss spectrophotometer), serum phosphorus 6.1 mg./100 ml.; serum alkaline phosphatase 13 KA units; blood urea 72 mg./100 ml.; her urinary calcium was 60 and 15 mg. per 24 hours in two determinations. She had not, it seemed, had excessive amounts of vitamin D. She continued to have thyroid extract. From September 14 she was given a preparation of milk with a low calcium content (Locasol, Trufood Ltd.). No vitamins were added. After 6 weeks of this treatment her serum calcium was still 13 mg./100 ml. Prednisone 5 mg. daily was added to the treatment on October 29 and with this the serum calcium fell to 10.8 mg./100 ml. after 3 weeks. Her appetite improved and she was no longer constipated. The investigations and treatment are summarized in Table II.

Between the age of 13 and 18 months the child did not receive thyroid extract. This was first to establish conclusively that she had a continued need for this treatment and secondly to see what effect, if any, the treatment had on her serum calcium. During the 5 months with-

out thyroid her weight increased by 1.2 kg. to 7.6 kg., her height increased by 8 cm. to 70 cm., and the circumference of her head increased by 0.5 cm. to 45 cm. No further teeth erupted (6 had done so), but the first carpal ossification centre appeared. At the end of the 5 months without treatment her skin was again dry and yellow, her face was puffy, she was apathetic and did not attempt to stand. She fed only reluctantly. Her physical and mental development corresponded to that of a child of 6-9 months. Her serum cholesterol was 400 mg./100 ml., PBI 1.7 μ g./100 ml. The thyroid uptake of ¹³¹I was very low, consistent with athyroid cretinism. On December 4, 3 months after treatment had been resumed with L-thyroxine, she looked quite different. The colour of her skin was normal; her face was no longer puffy; she was active and could stand and, with help, she could even walk; she fed herself; she spoke and understood a few words. Her weight had increased by 0.65 kg. and her length by 6 cm. She now had 12 teeth and a second carpal ossification centre had appeared. The serum cholesterol had fallen to 133 mg./100 ml. and the PBI had increased to 8.9 μ g./100 ml. (Table I).

Her physical development was retarded by about 9 months but her speech and social behaviour were retarded by only 3 months, and she was still developing rapidly. The serum calcium was 10.9 mg./100 ml. (s.g. 1,022) when treatment with thyroid was stopped; 10.8 (s.g. 1,025) on July 1, 1964, 11.0 (s.g. 1,026) on September 4, and 11.5 mg./100 ml. in December, 1964.

Cystathioninuria. When she was admitted the second time the urinary amino acids were examined because of her slow progress. The first chromatogram, on October 1, 1963, was normal except for a possible increase in glycine, but 7 days later, 3 weeks after beginning the treatment with Locasol, there was an abnormal spot in addition to the glycine spot. It was identified as cystathionine because (1) it ran with a cystathionine marker in 3 different solvents; (2) it disappeared after oxidation with peroxide; (3) it gave a positive test with iodoplatinate (Smith, 1960); and (4) it could be eluted in the position of cystathionine from a Moore and Stein ion-exchange column (Moore and Stein, 1954).

Fig. 2 shows the excretion of cystathionine on different days. Cystathionine was found when her urine was

TABLE II
Biochemical Changes Before, During, and After Treatment of Hypercalcaemia

Date	Weight (kg.)	Serum Ca (mg./100 ml.)	Serum P (mg./100 ml.)	Serum Alkaline Phosphatase KA Units	Urine Ca (mg./24 hr.)	Plasma Urea (mg./100 ml.)	Serum Proteins* (g./100 ml.)		Approx. Vitamin D Intake (i.u./day)	Treatment			
							Total	Albumin		Normal Diet	Farola	Locasol	Prednisone (5 mg. daily)
1963													
10 Sept.	4.4	13.9	6.1	13	—	72			375	†			
20	4.3	13.1	—	—	—	—			0				
30	4.2	12.7	6.9	—	—	—			0			▲	
4 Oct.		—	—	—	60	—			0				
5		—	—	—	15	—			0				
8		13.9	—	15	—	—			0				
11		—	6.1	—	—	—			0				
23	4.1	13.0	—	—	—	—			0				
29	4.1	—	—	—	—	54			0		▲		
7 Nov.	4.1	11.0	—	—	—	48	5.9		0				
13		11.2	4.4	22.5	—	48	5.9		0				
21		10.8	—	—	—	38	5.9		0				
29	4.3	11.3	—	—	—	42	6.3		0				
8 Dec.		—	—	—	15	—			0				
9		9.8	—	—	11.6	60	5.9		0				
17	4.2	10.0	—	—	—	45	4.7		0				
24		9.4	—	—	—	38	4.7		0				
1964													
1 Jan.	4.1	9.2	—	—	—	—	3.8	2.4	0				
10		10.0	—	—	—	30	4.3	2.8	0				
17	4.0	9.0	—	—	—	40	3.2		0	▲	▼	▼	
22		—	—	—	—	—	3.9	2.6					
29	4.0	9.3	—	—	—	15	3.6		400				
6 Feb.		10.3	—	—	—	—	5.1		400				
14	4.6	11.3	—	—	—	—	5.2	3.5	400				
19		11.2	—	—	—	—	6.1	3.9	400				
4 Mar.	5.7	11.4	—	—	—	—	6.3		400				
18		10.8	—	—	—	—	6.7		400				
20 May	7.2	10.9	—	—	—	35	5.5		400				
1 July	7.6	10.8	—	—	—	38	6.7		400				
2 Sept.		11.0	—	—	—	—	7.0		400	▼			Thyroid stopped

* Where total protein only is given it was calculated from the specific gravity of the serum; where albumin is also given values were obtained by the biuret method.
† γ globulin 390 mg./100 ml. (normal).

again examined on October 23 and November 1. On this day a 24-hour collection of urine contained 69 mg. cystathionine (normal 4.3 to 13.9 mg.), 73 mg. glycine (normal 12.4 to 106.6 mg.), 26 mg. β -amino-isobutyric acid (normal 4.7 to 28.7 mg.; normal values taken from

Carver and Paska, 1961). 'Farola' (J. Marshall Co.) was added to the diet on November 3; the excretion of cystathionine persisted (November 15 and December 3, 6, 8, and 9). Cysteine 1 g. given by mouth on December 5 did not influence the excretion of cystathionine; cystine

FIG. 2.—The effect of small amounts of pyridoxine on the excretion of cystathionine.

appeared in the urine. Specimens of urine from the mother, the father, and the three sisters were normal.

The infant's ability to metabolize tryptophan (900 mg. orally) was impaired. The 24-hour urinary excretion of xanthurenic acid increased from 2 mg. to 6.8 mg. These amounts are abnormally large (Bessey *et al.*, 1957). The excretion of taurine was less than 3 mg. and therefore abnormally small.

Effect of pyridoxine. A single intramuscular dose of 0.5 mg. pyridoxine was given on December 12. The first 12-hour specimen of urine after this injection contained only a trace of cystathionine and the next 24-hour collection none. On December 15 and 16 there was again a trace of cystathionine in the urine. From December 16-23 she had daily ascorbic acid 25 mg., vitamin A 2,500 units and Becosym Forte syrup (Roche) 5 ml., containing thiamine 5 mg., nicotinic acid 20 mg., riboflavin 2 mg., and pyridoxine 2 mg. Cystathionine had disappeared from the urine when it was first re-examined on December 19. The treatment provided a total dose of 14 mg. pyridoxine in 7 days. From December 23 she had the same vitamin supplements, in the same dose, with the exception of pyridoxine. The urine remained free of cystathionine until January 13 when traces reappeared, and by January 17, 1964, there were appreciable amounts. On January 23 she again received one dose of pyridoxine 0.5 mg. by intramuscular injection. The cystathionine took longer to disappear from the urine this time, but it was almost absent by January 29 and absent from February 3 onwards.

Meanwhile, the serum calcium had fallen to 9.0 mg./100 ml., but the plasma protein concentration was only 3.9 g./100 ml., albumin 2.5 g./100 ml. (Table II). In the 22 weeks from admission she had lost 0.3 kg. On January 29 her diet was changed to milk in the form of SMA, which now has pyridoxine added to it in the proportion of 0.09 mg. pyridoxine hydrochloride per oz. (28 g.) of the powder. When she was discharged on February 20 the total plasma protein concentration was 6.1 g., albumin 3.9 g./100 ml., serum Ca 11.2 mg./100 ml. (normal by the Zeiss flame spectrophotometer up to 11.3 mg./100 ml.).

Discussion

Hypercalcaemia may be encountered in both untreated and treated cretins. According to Royer (Royer *et al.*, 1958; Mathieu, Habib, Cuisinier, Muller, and Royer, 1961) the untreated patient absorbs excessive amounts of calcium which accumulate in the blood and in the bone. When the patients are treated with thyroxine the bone releases its abnormal calcium content and if the rate of release of calcium exceeds the capacity of the kidney to excrete it the plasma calcium rises (cf. Braid, 1951).

Our study does not provide any evidence on the mechanism of the hypercalcaemia. Our patient's clinical state was indistinguishable from that of

infants with 'idiopathic' hypercalcaemia; she had an 'elfin' facies and an aortic murmur, and one wonders whether in idiopathic hypercalcaemia these signs may be a result of hypercalcaemia rather than an independent feature of the disorder. The infant had not had excessive amounts of vitamin D.

The interest of this patient is her cystathioninuria and its relation to a simple deficiency of pyridoxine produced by an artificial feed selected for therapeutic reasons. Cystathioninuria first appeared 3 weeks after she had been put on a purified diet which lacked pyridoxine as well as other water-soluble vitamins. The manufacturers of Locasol inform us that in the preparation of Locasol or similar products the milk protein is precipitated and washed to rid it of minerals, and in this process the water soluble vitamins are removed. Only the minerals that are necessary to redissolve the protein are replaced.

The abnormal excretion of cystathionine was corrected by a normal intake of pyridoxine and was probably a direct effect of pyridoxine deficiency. The other cases of cystathioninuria that have been described required more than the normal intake of pyridoxine to abolish the cystathioninuria.

This child's transient deficiency of pyridoxine did not result in any apparent mental retardation. Mental retardation does complicate the convulsions of pyridoxine deficiency and of pyridoxine dependency, unless they are treated at a very early stage (Waldinger and Berg, 1963; Waldinger, 1964).

Twice when 0.5 mg. pyridoxine was given by intramuscular injections, cystathionine disappeared from the urine. After the infant had received, during one week, 14 mg. pyridoxine by mouth, the cystathionine disappeared from the urine for at least 25 days. It is not possible to say how much of the injected pyridoxine was lost by excretion in the urine, or how much of the ingested pyridoxine was not absorbed. The observations suggest that the oral requirement was about 0.5 mg. daily and within the normal range of 0.2-0.5 mg. daily suggested by Bessey *et al.* (1957).

Cystathioninuria may be an early sign of pyridoxine deficiency. It should, therefore, be sought when pyridoxine deficiency is suspected, for instance in infants with unexplained fits.

Summary

In an infant cretin the serum calcium rose to over 13 mg./100 ml. when she was treated with thyroid.

For her hypercalcaemia she received a purified milk powder which contained no calcium but also no water-soluble vitamins.

After three weeks cystathionine appeared in the

urine. There was other biochemical evidence of pyridoxine deficiency and the cystathionine twice disappeared when the child received 0.5 mg. of pyridoxine.

The appearance of cystathionine in the urine is a sign of simple pyridoxine deficiency in infancy.

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