A CASE OF HISTIDINAEMIA

BY

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In 1961, Ghadimi, Partington and Hunter reported increased levels of histidine in both the urine and plasma of two sisters. This was followed in 1962 by a report from Auerbach, DiGeorge, Baldridge, Tourtellotte and Brigham of a girl with increased histidine levels. The urine of all three cases gave a reaction to ferric chloride similar to that seen in phenylketonuria, but chromatographic studies indicated that histidine was the only amino acid being excreted in excess. Auerbach *et al.* (1962) showed in their case a defect in the conversion of histidine to urocanic acid, probably due to a lack of histidase activity.

This report concerns a case of histidinaemia associated with recurrent infections, retarded growth and a slight degree of mental retardation in a boy with brown hair and brown eyes.

Case Report

This infant was born three weeks prematurely in 1956, following a normal pregnancy. His birth weight was 4 lb. 7 oz. (1.93 kg.). He was first seen at this hospital at the age of 9 months with a pyrexia of unknown origin, his weight then being 12 lb. 7 oz. (5.66 kg.). The pyrexia subsided spontaneously and it was considered that his slow weight gain was the result of feeding mismanagement and a most unsatisfactory home environment. At 17 months he was readmitted with a rash and fever which subsided within a week.

Haematological investigations gave the following results: Haemoglobin 7.1 g./100 ml., white cell count 30,000/c.mm., neutrophils 74%, lymphocytes 24% and monocytes 2%; platelets 300,000/c.mm. The anaemia was considered to be of nutritional origin, and treatment with iron and folic acid was commenced, which was to be continued at home. However, six weeks later he was readmitted with fever and vomiting and a haemoglobin of 7.0 g./100 ml. The iron and folic acid therapy was continued for three weeks (on the assumption that this had not been carried out at home), but as this produced no rise in the haemoglobin a blood transfusion was given and he was discharged two weeks later. The haemoglobin was then 14.6 g./100 ml. with a normal white cell count. Since then there has been no recurrence of anaemia.

For the next 18 months he was seen occasionally in the out-patients' department, during which time he

suffered various infections and vomited frequently. At 3 years and 7 months he weighed only 21 lb. (9.52 kg.) and was admitted for investigation. Serum electrolytes, calcium, phosphate, alkaline phosphatase, cholesterol and blood urea were normal. Serum protein electrophoresis showed a normal pattern. Microscopic examination of the faeces and urine showed no abnormality, but a chromatogram of the urine indicated that he was excreting large amounts of histidine. Radiological studies indicated normal bone development. It was observed that there were definite signs of mental retardation, but no explanation could be given for the growth failure.

At 4 years of age he was admitted with fever and convulsions, the result of a right otitis media. This responded well to antibiotic treatment. Histidine excretion was again found to be raised. In the next year he was seen in the out-patients' department several times with various minor infections. A course of norethandrolone, 10 mg./day, was given for one month, but there was no growth response. A second course of norethandrolone, 10 mg./day, was given for one month at 5 years of age, but again no growth response was observed.

The last admission was in 1962, at the age of 5 years and 5 months, for investigation of the cause of the histidinuria. His weight was then 28 lb. (12.7 kg.) and his height 39 in. (99 cm.), both measurements being below the 10 percentile for his age.

Methods

Urine. Urine was desalted on a Dowex 50 $\times$ 8 column (Smith, 1958) and then chromatographed on Whatman No. 1 paper. For identification two-dimensional chromatography was performed using three different solvent systems: (1) butanol-acetic acid-water (120 : 30 : 50) followed by phenol-ammonia; (2) pyridine-acetone-ammonium hydroxide (5 : 3 : 2) followed by isopropyl alcohol-formic acid-water (8 : 1 : 1); (3) butanol-acetic acid-water (120 : 30 : 50) followed by butanol-pyridine-water (1 : 1 : 1).

The chromatograms were dipped either in 0.5% ninhydrin in acetone, sulphanic acid (Pauly) reagent or anisidine reagent.

For semiquantitative estimations of histidine, known volumes of the desalted urines were chromatographed in butanol-acetic acid-water (120 : 30 : 50), stained with Pauly's reagent and compared with known amounts of histidine run on the same paper.

Two-dimensional chromatograms for urinary indoles were run in the system isopropyl alcohol-ammonia-water (200 : 10 : 20) followed by butanol-acetic acid-water (120 : 30 : 56). The chromatograms were examined with ultraviolet light and then dipped in Ehrlich reagent.

Plasma. Blood was collected into heparinized bottles. The plasma was separated by centrifugation and deproteinized with 20% perchloric acid. The supernatant was passed through a Dowex 50 × 8 column and the amino acids eluted with 2N ammonium hydroxide. The eluate was taken to dryness, redissolved in water and the histidine estimated as described above for urine.

Clearance Test. The patient was fasted overnight and from 6 a.m. onwards was given 50 ml. water to drink every half hour. Urine voided at 7 a.m. was discarded and all urine for the next three hours was collected. Two blood samples were collected, one fasting and one half-way through the urine collection. Urine and plasma histidine concentrations were measured and the clearance rate determined according to the formula (Cusworth and Dent, 1960),

$$\text{Clearance} = \frac{\mu\text{g./min.} \times 1.73 \text{ m}^2}{\mu\text{g./ml.}}$$

Load Test. The patient was fasted overnight and from 6 a.m. onwards was given 50 ml. water to drink every half hour. Urine voided at 7 a.m. was discarded and the child then given a dose of L-histidine (100 mg./kg. body weight = 1.3 g. L-histidine) in 50 ml. orange juice. Urine was collected for the next three hours. Blood was collected before the dose and one and two hours after.

Results

Urine. All urine samples showed raised levels of histidine, but normal levels of other amino acids. The histidine present in two 24-hour collections was 312 and 578 mg. respectively compared with values from four normal children of 30–80 mg./24 hours. In addition, three other Pauly positive (red) spots were observed, but these have not as yet been conclusively identified. Excretion of indoles was normal in all specimens. Urine specimens from both parents and two siblings showed normal excretion of histidine, other amino acids and indoles.

Plasma. The fasting level on two occasions was 9 mg./100 ml., compared with quoted normal values of approximately 1 mg./100 ml. (Stein and Moore, 1954; Ghadimi *et al.*, 1961). No other Pauly positive (red) spots were detected on plasma chromatograms.

Clearance Test. Both the fasting plasma and that collected during the test contained 9 mg./100 ml. histidine. Urinary excretion was 60.2 mg./3 hours.

Histidine clearance = $\frac{960 \times 90}{1000} = 10.7 \text{ ml./min.} \times 1.73 \text{ m}^2$ compared to values found by Cusworth and Dent (1960) of 4.7–9.7 in normal adults.

Load Test. Urinary excretion of histidine was 45 mg./3 hours. Fasting plasma level was 9 mg./100 ml. The levels of histidine at one hour and two hours after the dose were 20 mg./100 ml. and 17 mg./100 ml. respectively.

Ferric Chloride Test. All urine specimens were tested with ferric chloride and 'phenistix', but the only positive reaction was given by the urine collected during the load test. This gave a strong green colour, similar to that seen in phenylketonuria.

Discussion

As both the plasma level and the urinary excretion of histidine are considerably above normal, the histidinuria in this case is suggestive of the 'overflow' type. This is further supported by the fact that the histidine clearance rate is in close agreement with that found by Cusworth and Dent (1960) for normal adults, indicating normal renal transport of histidine in this case.

The oral load tests indicated a rapid absorption of histidine from the gut, but only slow removal from the plasma.

It is of interest that urine from the patient only showed a positive reaction with ferric chloride during the load test in contrast to the other three reported cases in whom positive reactions were obtained from random urine samples.

Two-dimensional chromatograms of the urine showed three other distinctly separated Pauly positive (red) spots besides histidine, two of which are considered to be imidazole acetic acid and imidazole lactic acid, but the identity of the other spot is uncertain. Ghadimi, Partington and Hunter (1962) reported six Pauly positive spots, four of which they identified as imidazole acetic acid, imidazole lactic acid, imidazole pyruvic acid and histidine. Of the other two spots, one was unidentified and the sixth was thought to be a second spot due to histidine. Auerbach *et al.* (1962) reported four positive spots and identified them as imidazole acetic acid, imidazole lactic acid and two spots for histidine. In the case reported in this paper it is considered that there are three spots besides histidine, because on one-dimensional chromatograms, although occasionally there was a splitting of the histidine spot, there were always three other well-separated spots. The possibility that the fourth spot in this urine could be imidazole pyruvic acid is doubtful for two reasons. First, this substance

TABLE

Case				Height	Weight	Recurrent Infections	Speech	Mental Status
Ghadimi <i>et al.</i> (1961)	Case 1	..	..	Normal	Normal	—	Slurred	Normal
(1962)	Case 2	..	..	Normal	Normal	—	Normal	Normal
Auerbach <i>et al.</i> (1962)	..	..	..	Below normal	Below normal	—	Slurred	Normal
Propositus	..	..	..	Below normal	Below normal	—	Normal	Retarded

yields only a faint colour with Pauly reagent (Ghadimi *et al.*, 1962) and secondly, Ghadimi *et al.* have shown that imidazole pyruvic acid is responsible for the positive ferric chloride test which is only given in our case by the urine collected during the load test.

Bessman and Baldwin (1962) reported three families with cerebro-macular degeneration and imidazole aminoaciduria, but normal blood levels of imidazole compounds. Their results suggest a renal abnormality rather than a specific enzyme defect. The results of the investigation in the present case suggest that there is a specific defect in the metabolism of histidine probably at the level of histidase activity. Thus this case appears to be similar to those described by Ghadimi *et al.* (1961) and Auerbach *et al.* (1962) and not to those described by Bessman and Baldwin (1962).

The Table summarizes the outstanding clinical features of all the cases reported.

From this Table it is seen that there is no common clinical feature. In the case reported here, the most obvious feature is the gross failure to grow normally, which was unaffected by anabolic steroid therapy on two occasions. However, it is obviously not possible as yet to ascribe any particular symptom to the abnormality in histidine metabolism.

Summary

A boy with slight mental retardation, recurrent infections and pronounced failure to grow, has been shown to excrete large amounts of histidine and other imidazole-like compounds.

Plasma levels, clearance rate and load tests indicate that the histidinuria is of the 'overflow' type.

It is postulated that the basic disturbance in histidine metabolism is a lack of histidase activity.

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