Increased Excretion of 5-Hydroxyindoleacetic Acid in Urine of Children with Untreated Coeliac Disease

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Challacombe, D. N., Brown, G. A., Black, S. C., and Storrie, M. H. (1972). *Archives of Disease in Childhood*, 47, 442. **Increased excretion of 5-hydroxyindoleacetic acid in urine of children with untreated coeliac disease.** 24-hour urine excretions of 5-hydroxyindoleacetic acid (5HIAA) have been measured in 13 children with coeliac disease before treatment. All were excreting more 5HIAA than an age-matched group of 22 control children. On introduction of a gluten-free diet clinical remission was accompanied by decreasing 5HIAA excretion. Excretion was normal in cases of acute and chronic diarrhoea and cystic fibrosis.

The test is proposed as an aid to diagnosis and a method of monitoring satisfactory dietary control.

Raised blood levels of serotonin have been reported in adults with coeliac disease (Pimparkar, Senesky, and Kalser, 1961; Warner and Cohen, 1962). Serotonin (5-hydroxytryptamine, 5HT), a metabolite of tryptophan, is produced principally by argentaffin cells in the gastrointestinal tract. It is metabolized by tissue monoamine oxidase to 5-hydroxyindolylaldehyde which is further oxidized and excreted in the urine as 5-hydroxyindoleacetic acid (5HIAA). The excretion of 5HIAA in the urine of adults with untreated coeliac disease has also been shown to be raised by some authors (Haverback and Davidson, 1958; Kowlessar *et al.*, 1958; Haverback, Dyce, and Thomas, 1960; Pimparkar *et al.*, 1961; Scriver, 1961; Slesinger, 1961; Kowlessar, Haeflner, and Benson, 1964; Benson, Kowlessar, and Slesinger, 1964), though not by others (Connell, Rowlands, and Wilcox, 1960). In those cases where the introduction of a gluten-free diet resulted in clinical and biochemical remission, the levels of blood serotonin (Pimparkar *et al.*, 1961) and urinary 5HIAA (Slesinger, 1961; Kowlessar *et al.*, 1964; Benson *et al.*, 1964) returned to normal.

Comparable studies in children with coeliac disease have not been reported previously.

Materials and Methods

24-hour urinary excretion of 5HIAA was estimated in 13 children with untreated coeliac disease aged between

6 months and 2 years. The observations were repeated at varying intervals in 8 of these children after the introduction of a gluten-free diet. The diagnosis was confirmed in each case by the demonstration of a flat duodenal mucosa obtained by peroral biopsy, and by the occurrence of clinical remission after the gluten-free diet. We also investigated a group of 8 patients with other gastrointestinal disorders (Table) for evidence of abnormal 5HIAA excretion. Control data were obtained from 22 children of approximately equal age, in hospital for investigation of disorders unconnected with the gastrointestinal tract.

All serotonin-containing foods (Erspamer, 1966) and all drugs were excluded from the diet for 24 hours before, and during, the urine collection.

The urine was collected directly into 10 ml glacial acetic acid and stored at -20°C until analysed.

5HIAA was measured by a method based on that of Macfarlane *et al.* (1956), with improvements suggested by Mustala, Tuomisto, and Airaksinen (1964). Each urine was analysed in duplicate with added 5HIAA in the second analysis. Recovery of the added 5HIAA was calculated and each analysis figure was corrected using the percentage recovery found. In a series of thirty 24-hour urine specimens, mean percentage recovery of added 5HIAA was 89%, with a range (± 2 SD) of 78.2–99.7%.

Collection of reliable 24-hour urine specimens was difficult in the age range of children investigated, and the data were therefore expressed in relation to creatinine excretion. Creatinine was measured by a standard Technicon Auto-Analyser alkaline picrate procedure (Methodology sheet N-11b) with a urine dilution step incorporated in the manifold.

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Excretion of 5-Hydroxyindoleacetic Acid in Children with Untreated Coeliac Disease 443

TABLE
Urinary 5HIAA : Creatinine in Control Patients,
Coeliac Disease, and Various Gastroenterology
Disorders

Diagnosis	Sex	Age	μg 5HIAA/mg Creatinine
<i>Controls (22 cases)</i>			
Anaemia	F	2 wk	14.6
Congenital heart disease	M	2 wk	15.7
Congenital heart disease	M	2 wk	20.9
Tracheo-oesophageal fistula	M	3 wk	13.1
Congenital heart disease	M	1 mth	6.5
Congenital heart disease	M	6 wk	23.0
Post meningitis	M	2 mth	16.9
Inguinal hernia	M	2 mth	13.1
Chest infection	M	4 mth	20.6
Otitis media	F	6 mth	15.5
Chest infection	M	6 mth	18.0
Upper respiratory tract infection	M	7 mth	19.4
Upper respiratory tract infection	M	9 mth	15.3
Normal	M	9 mth	14.5
Tracheal stricture	M	9 mth	16.7
Normal	M	1 yr	10.2
Normal	M	1 yr 2 mth	10.4
Asthma	M	1 yr 3 mth	10.6
Normal	M	1 yr 6 mth	11.0
Post febrile convulsions	M	1 yr 8 mth	9.6
Asthma	M	2 yr 1 mth	12.0
Chest infection	M	2 yr 2 mth	8.3
<i>Coeliac disease (13 cases)</i>			
	F	4 mth	25.2
	F	6 mth	43.0
	F	9 mth	22.5
	F	10 mth	38.0
	F	11 mth	14.2
	M	1 yr	19.3
	F	1 yr 2 mth	33.0
	F	1 yr 2 mth	15.1
	F	1 yr 4 mth	26.0
	M	1 yr 4 mth	18.0
	F	1 yr 5 mth	23.0
	F	1 yr 9 mth	20.7
	M	2 yr 4 mth	23.6
<i>Miscellaneous (8 cases)</i>			
Fibrocystic disease	M	1 yr	11.7
Fibrocystic disease	M	1 yr 5 mth	10.8
Acute diarrhoea	M	5 wk	20.0
Acute diarrhoea	M	4 mth	18.6
Acute diarrhoea	M	2 yr 1 mth	4.6
Chronic nonspecific diarrhoea	M	2 mth	13.3
Chronic nonspecific diarrhoea	F	11 mth	10.9
Fibrocystic disease and coeliac disease	F	1 yr 7 mth	23.8

Nominal 24-hour excretions of 5HIAA were expressed as μg 5HIAA/mg creatinine.

Results

The values of the 5HIAA : creatinine ratio in relation to age in the patients with coeliac disease and control children are shown in Fig. 1 and the Table. The value of this ratio is high in early infancy, falling to a lower level at 1 year. The

high initial value of the ratio is due to an increased rate of 5HIAA excretion in early infancy and coincides with the rising levels of whole blood 5HT occurring during this period (Hazra, Benson, and Sandler, 1965). The subsequent decrease in ratio is not due to an absolute decrease in 5HIAA excretion, but to a reduced increment rate, accompanied by a constantly increasing creatinine excretion rate. Between 1 year and 2 years 2 months, the upper limit of our age group, the ratio remains constant.

Although ratios in some untreated coeliac children over 1 year of age were lower than values in younger controls, when compared to controls of appropriate age, no overlap of these ratios occurred. Notwithstanding the overlap introduced by the age factor, the mean value for the ratio in the coeliac children was greater ($P = 0.001$) than the mean for the control group. After the introduction of a gluten-free diet (Fig. 2), the ratio decreased in all 8 cases in whom follow-up studies were performed. In half of the cases the ratio fell to levels indistinguishable from control values, but in the other half, values were still above the normal controls. It is possible that after a longer period of treatment all 8 values would have returned to normal. After treatment the ratios fell to 39–89% of their pretreatment value, with an average fall for the 8 patients to 63%. The fall in ratio on treatment was entirely due to decreased 5HIAA excretion. Urinary creatinine values, before and after the introduction of a gluten-free diet, were unchanged. The 5HIAA : creatinine ratio in the small group of other gastrointestinal disorders did not exceed control values for their age, with the exception of one child with both coeliac disease and fibrocystic disease. In this patient the value was within the high range characteristic of patients with coeliac disease. Two weeks after a gluten-free diet the ratio had fallen to a normal value.

Discussion

The increased excretion of 5HIAA in the urine of children with untreated coeliac disease agrees with previously reported findings in adults with this disorder. The cause of increased 5HIAA excretion is unknown. The metabolic pathway leading to the production of 5HIAA has been studied in adults by L-tryptophan loading tests, and an augmented shunt of the 5HT to 5HIAA pathway shown in the untreated patients (Kowlessar *et al.*, 1964). Raised levels of whole blood 5HT have also been reported in adults with untreated coeliac disease and these fell to normal after a gluten-free diet (Pimparkar *et al.*, 1961). Though

FIG. 1.—5HIAA: creatinine ratios in 24-hour urine collections from children with untreated coeliac disease (○), and age-matched controls (●).

comparative studies of whole blood 5HT levels have not been performed in children, our data suggest that it would be similarly abnormal in children with untreated coeliac disease.

Increased urinary excretion of other tryptophan metabolites, such as indole-3-acetic acid and indican, have also been reported in coeliac disease (Haverback *et al.*, 1960; Kowlessar *et al.*, 1964). The raised

levels of these metabolites probably result from an abnormal small intestinal flora, or a normal colonic flora acting on unabsorbed proteins or amino acids (Kowlessar *et al.*, 1964; Fordtran, Scroggie, and Potter, 1964). The administration of tetracycline to adults with untreated coeliac disease lowered the urinary excretion of indole-3-acetic acid and indican, but did not affect the raised levels of 5HIAA (Kowlessar *et al.*, 1964). As urinary 5HIAA excretion was also unaffected by tryptophan or serotonin infusion into the colon, the increased excretion of 5HIAA in coeliac disease is probably unrelated to colonic bacterial flora (Fordtran *et al.*, 1964).

An increased rate of 5HT breakdown resulting from increased tissue monoamine oxidase activity might also explain the increased urinary levels of 5HIAA. The enzyme has been estimated and found to be low in the duodenal mucosa and normal in plasma (Challacombe, Sandler, and Southgate, 1971), and it seems unlikely therefore that increased monoamine oxidase activity is primarily responsible for the increased urinary excretion of 5HIAA. Since the excretion of 5HAA decreases on withdrawal of dietary gluten, gluten itself may have a direct effect on the argentaffin cells resulting in increased release of 5HT. As published family studies of coeliac disease (Carter, Sheldon, and Walker, 1959) suggest that the incidence of this disease is greater among relatives of affected individuals than in the general population, the

FIG. 2.—5HIAA: creatinine ratios in 8 patients with coeliac disease before and during treatment with a gluten-free diet.

possibility of an inborn abnormality of argentaffin cell metabolism in this disease must also be considered.

Though our investigations in other gastrointestinal disorders suggest that raised 5HIAA excretion is a specific manifestation of coeliac disease, a non-specific association with villous atrophy has not yet been excluded. Further work on 5HIAA excretion in other gastrointestinal disorders (Ammann, 1965) characterized by villous atrophy will also be necessary.

The measurement of 5HIAA : creatinine ratio in 24-hour urine collections may prove to be a useful aid in the laboratory diagnosis and dietary management of children with coeliac disease. Though the collection of a 24-hour specimen of urine is difficult in this age group, it may be possible to use the 5HIAA : creatinine ratio from urines collected over shorter intervals of time.

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