Hydroxyproline excretion in infantile gastroenteritis

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SUMMARY Total hydroxyproline/creatinine ratio (THP:Cr) was measured in random urine specimens from 18 infants with gastroenteritis. THP:Cr was sometimes increased during the acute phase of the illness and the reasons for this are discussed. Seven patients failed to thrive after the acute phase and THP:Cr was low in all of these. The test appears to be a sensitive indicator of the onset of failure to thrive, and it is suggested that it may be of value in the management of these patients.

Infantile gastroenteritis is sometimes followed by persistent diarrhoea and failure to thrive. This is often attributed to malabsorption caused by carbohydrate intolerance (Clarke *et al.*, 1964; Burke *et al.*, 1965; Lifshitz *et al.*, 1971; Bartrop and Hull, 1973). The condition may persist for weeks or months with growth failure and cachexia. Management usually involves the administration of various synthetic diets, and accurate assessment of the response to these would be of use in deciding their therapeutic value in each individual case.

Hydroxyproline is largely a breakdown product of collagen, and its rate of excretion is used as a measure of the rate of collagen turnover. Total hydroxyproline excretion has been shown to be a sensitive indicator of growth rate in normals (Younoszai *et al.*, 1967; Zorab *et al.*, 1970; Wharton *et al.*, 1973) and various types of growth failure (Whitehead, 1965; Wharton *et al.*, 1972; Teller *et al.*, 1973).

The purpose of this investigation was to measure total hydroxyproline excretion, expressed as total hydroxyproline:creatinine ratio (THP:Cr), in cases of failure to thrive after gastroenteritis and to determine whether it is a useful indicator of progress.

Methods

Failure to thrive was defined as persistence of diarrhoea and failure to gain weight for 10 days after the onset of gastroenteritis in infants. THP:Cr was

measured serially in random samples of urine from 18 inpatients with gastroenteritis. Thirteen patients were studied in the acute phase; 11 of these recovered uneventfully, but two failed to thrive. A further five patients failed to thrive but were not examined in the acute phase. Each patient was compared with a normal control whose age did not differ from his own by more than 15%. Patients and controls abstained from meat and gelatin for 24 hours before samples were collected. The specimens were placed in store at -20°C within two hours of voiding.

Urinary total hydroxyproline was measured by the method of Goverde and Veenkamp (1972). A hot air oven with a fan giving uniform internal temperature was used for the hydrolysis stage. The means of duplicate total hydroxyproline measurements were taken, and these were in the range 0.17-30.7 mmol/l. Standard deviations of the means were 0.2-6 mmol/l, mean 0.38 mmol/l. Urinary creatinine was measured by the method of Bonsnes and Tausky (1945) modified for the Technicon AutoAnalyzer. THP:Cr was expressed as millimoles hydroxyproline per mole of creatinine.

Results

THP:Cr in nine of the 13 patients seen in the acute phase rose to levels exceeding the 90th centile for normals established by Wharton *et al.* (1972). The peak occurred four to nine (median 5) days from the onset of symptoms. When the highest values seen in the 13 patients were compared with their controls, using the paired *t* test, they were found to be significantly higher ($p < 0.01$). The individual values and ages of this group are shown in Table 1.

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Table 1 *Ages and levels of urinary total hydroxyproline/creatinine ratio (THP:Cr) in patients with gastroenteritis seen during the acute phase*

Name	Sex	Age	Urinary THP:Cr (mmol/mol)										Control	90th centile for normals
			2	3	4	5	6	7	8	9	10			
RM	F	1 mth	665		362		525					452	1300	
TJ	F	4 "		404		43		767				533	608	
MG	F	5 "				462		694				287	444	
RP	M	6 "	268		238	243				217		248	288	
TP	M	9 "			235		164					308	257	
MD	F	14 "				175				422		156	236	
LH	M	14 "			735		96		88			203	183	
PM	M	18 "			330	217						191	152	
SH	M	2½ yr			95				825		125	171	137	
NL	F	2½ "	202		356		169					190	137	
MA	M	2½ "				452		249			205	116	137	
MH	M	3 "		67	85							177	139	
KU	F	6 "	102		104	122						172	87	

Conversion: SI to traditional units: THP:Cr 1 mmol/mol = 1.15 mg/g

THP:Cr was found to be lower than the paired controls in every case of failure to thrive, the difference being statistically significant using the paired *t* test ($p < 0.01$). THP:Cr was also lower than the 10th centile for normals (Wharton *et al.*, 1972) in each case. The individual values and ages of this group are shown in Table 2. The lowest values were recorded between five days and 10 weeks (median 16 days) after the onset of gastroenteritis.

Table 2 *Urinary total hydroxyproline/creatinine ratios (THP:Cr) in patients failing to thrive after gastroenteritis and later in the same patients when thriving*

Name	Age at onset of symptoms	Lower limit of normal THP:Cr (mmol/mol) for age	THP:Cr (mmol/mol)	
			Failing to thrive	Thriving
JO	7 days	434	217, 338	712
JH	2 weeks	573	377, 388	1887, 1380
WH	8 "	408	322	494, 611, 527, 1210
MF	3 months	303	46	485
VV	3 "	303	75	273*, 234*

*These specimens were taken three months after the first when the lower limit of normal THP:Cr would be 186 mmol/mol.

Conversion: SI to traditional units—THP:Cr: 1 mmol/mol = 1.15 mg/g

In five patients, THP:Cr was measured during the period of failure to thrive and later when the patient was gaining weight. The levels were significantly higher in the convalescent group ($p < 0.05$ using the paired *t* test). Figure 1 shows two cases of failure to thrive in which high readings during the acute phase were followed by low readings at the start of the period of failure to thrive.

In two patients, THP:Cr was measured just before they started to thrive. The ratios were low during

Fig. 1 Two patients showing high levels of THP:Cr during the acute phase of gastroenteritis and subsequently developing low levels when they entered a phase of failure to thrive: 10th and 90th centiles for normals are taken from Wharton *et al.* (1972).

failure to thrive and rose to normal when only a small weight gain had occurred (Fig. 2).

Figure 3 shows THP:Cr in 19 normal South London children superimposed on the 10th and 90th

Fig. 2 Two patients showing low levels of THP:Cr when failing to thrive followed by a rise to normal before a definite weight gain is seen. The 10th centiles for normals are taken from Wharton *et al.* (1972).

centiles for normal Bristol children reported by Wharton *et al.* (1972).

Discussion

The use of random specimens of urine has obvious practical advantages over 24-hour collections in infants. THP:Cr in 24-hour specimens of urine has been shown to be a more accurate measure of growth rate than total hydroxyproline alone (Allison *et al.*, 1966), and further studies have shown a reasonable correlation of this ratio in random specimens with that in 24-hour collections (Howells *et al.*, 1967; Younoszai *et al.*, 1969). However, Mautalen (1974) has pointed out that hydroxyproline excretion and THP:Cr in adults is subject to diurnal variation and has questioned the validity of measurements on random specimens. Reviewing this controversy, Wharton (1974) noted that the variations in THP:Cr in children were small enough to make measurements on single specimens adequate for clinical purposes. As an added safeguard, he suggested that

serial specimens should be collected at the same time of day. The normal values for Bristol children (Wharton *et al.*, 1972) were estimated on random specimens of urine.

It is often difficult to interpret small weight changes in children failing to thrive because of minor alterations in fluid balance. It is necessary to observe progressive weight gain over several days before it can be confidently stated that growth is restored. THP:Cr has potential advantages in giving early evidence of restored growth because it gives an indication of growth rate at a point in time. This is illustrated in Fig. 2 where the THP:Cr ratios were low during failure to thrive and rose to normal when there was still clinical doubt whether growth was restored on the basis of weight recordings. At this time one patient was only 25 g above the weight on admission (1800 g) and 100 g above the lowest weight. The other patient was 125 g above the weight on admission (4800 g) and 150 g above the lowest weight. The investigation may also be valuable in the early recognition of patients who fail to thrive after gastroenteritis, as shown by the low THP:Cr illustrated in Figure 1.

Formal and repeated tests of intestinal function are often impossible or inappropriate in these frail infants. Treatment is largely empirical, and several different synthetic diets may have to be tried in an individual patient before growth is restored. The results suggest that THP:Cr, being a more sensitive indicator of growth rate than weight measurements, may enable earlier selection of an effective diet.

The pathogenesis of failure to thrive after gastroenteritis is not well understood. A reduction in protein synthesis or an increase in catabolism might be important. Although we did not measure 24-hour hydroxyproline excretion, it seems likely that the low THP:Cr is caused by reduced hydroxyproline excretion. Since hydroxyproline is largely a breakdown product of collagen, the results suggest no significant increase in the catabolism of this protein. A failure of synthesis seems more likely.

High levels of THP:Cr were sometimes seen in the acute phase of gastroenteritis and are not easily explained. It seems unlikely that tissue collagen could be broken down fast enough to provide this rapid rise and fall in hydroxyproline excretion. The catabolism of hydroxyproline-containing plasma proteins, such as the complement component C1q (Reid *et al.*, 1972), seems a more plausible explanation. Alternatively, reduced creatinine excretion would result in increased THP:Cr. We have not yet explored these possibilities. In any event, the findings give useful background knowledge when following individual patients with gastroenteritis from the start of their illness.

Fig. 3 THP:Cr in 19 normal South London children used as controls superimposed on the 10th and 90th centiles for normals reported by Wharton et al. (1972).

Failure to thrive after gastroenteritis is often a serious condition and management may be hampered by difficulties in assessment of progress. It is suggested that THP:Cr may be a useful indicator of progress and that a larger study is justified.

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References

- Allison, D. J., Walker, A., and Smith, Q. T. (1966). Urinary hydroxyproline:creatinine ratio of normal humans at various ages. *Clin. chim. Acta*, **14**, 729-734.
- Bartrop, R. W. and Hull, D. (1973). Transient lactose intolerance in infancy. *Arch. Dis. Childh.*, **48**, 963-966.
- Bonsnes, R. W. and Taussky, H. H. (1945). On the colorimetric determination of creatinine by the Jaffe reaction. *J. biol. Chem.*, **158**, 581-591.
- Burke, V., Kerry, K. R., and Anderson, C. M. (1965). The relationship of dietary lactose to refractory diarrhoea in infancy. *Aust. paediat. J.*, **1**, 147-160.
- Clarke, J. T., Quillian, W., and Shwachman, H. (1964). Chronic diarrhea and failure to thrive due to intestinal disaccharidase insufficiency. *Pediatrics*, **34**, 807-813.
- Goverde, B. C. and Veenkamp, F. J. N. (1972). Routine assay of total urinary hydroxyproline based on resin-catalysed hydrolysis. *Clin. chim. Acta*, **41**, 29-40.
- Howells, G. R., Wharton, B. A., and McCance, R. A. (1967). Value of hydroxyproline indices in malnutrition. *Lancet*, **1**, 1082-1083.
- Lifshitz, F., Coello-Ramirez, P., Gutierrez-Topete, G., and Cornado-Cornet, M. C. (1971). Carbohydrate intolerance in infants with diarrhea. *J. Pediatr.*, **79**, 760-767.
- Mautalen, C. A. (1974). Circadian rhythm of the total hydroxyproline:creatinine ratio in urine. *Clin. Sci. molec. Med.*, **46**, 419-421.
- Reid, K. B. M., Lowe, D. M., and Porter, R. R. (1972). Isolation and characterization of Clq, a subcomponent of the first component of complement, from human and rabbit sera. *Biochem. J.*, **130**, 749-63.
- Teller, W. M., Genscher, U., Burkhardt, H., and Rommel, K. (1973). Hydroxyproline excretion in various forms of growth failure. *Arch. Dis. Childh.*, **48**, 127-132.
- Wharton, B. A. (1974). Circadian rhythm of the total hydroxyproline:creatinine ratio in urine (Letter). *Clin. Sci. Molec. Med.*, **46**, 420-421.
- Wharton, B. A., Gough, G., and Pennock, C. A. (1973). The relationship of height and weight velocities of adolescent boys to the total hydroxyproline:creatinine ratio in their random urines. *Clin. Sci.*, **44**, 359-365.
- Wharton, B. A., Gough, G., Williams, A., Kitts, S., and Pennock, C. A. (1972). Urinary total hydroxyproline:creatinine ratio. Range of normal, and clinical application in British children. *Arch. Dis. Childh.*, **47**, 74-79.
- Whitehead, R. G. (1965). Hydroxyproline creatinine ratio as an index of nutritional status and rate of growth. *Lancet*, **2**, 567-570.
- Younoszai, M. K., Andersen, D. W., Filer, L. J., Jr.,

- and Fomon, S. J. (1967). Urinary excretion of endogenous hydroxyproline by normal male infants. *Pediat. Res.*, **1**, 266-270.
- Younoszai, M. K., Kacic, A., Dilling, L., and Haworth, J. C. (1969). Urinary hydroxyproline-creatinine ratio

- in normal term, pre-term and growth-retarded infants. *Arch. Dis. Childh.*, **44**, 517-520.
- Zorab, P. A., Clark, S., Harrison, A., and Seel, J. R. (1970). Hydroxyproline excretion and height velocity in adolescent boys. *Arch. Dis. Childh.*, **45**, 763-765.

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