Preliminary Communications

Dipeptide Absorption in Cystinuria

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Summary: Absorption of a dipeptide and its constituent amino-acids has been studied by a perfusion technique in a patient with cystinuria and in a normal subject. Though the cystinuric patient was unable to absorb the dibasic amino-acid lysine in its free form, the dipeptide glycyl-L-lysine was well absorbed. Both free lysine and glycyl-L-lysine were well absorbed in the normal subject.

This finding suggests that the reason that clinical malnutrition does not occur in cystinuria, despite defective intestinal absorption and heavy renal loss of dibasic amino-acids and cystine, is that these amino-acids can be absorbed in the form of oligopeptides.

INTRODUCTION

Patients suffering from cystinuria show no clinical evidence of malabsorption, despite defective intestinal absorption of the dibasic amino-acids and cystine together with excessive loss of these amino-acids from the kidney. It is possible that though such subjects are unable to absorb the free amino-acids normally they can nevertheless absorb a sufficient quantity to avoid developing overt malnutrition. An alternative explanation might be that though the amino-acids are not absorbed in their free state they are able to cross the intestinal mucosa in the form of oligopeptides.

Recently increasing evidence has suggested that absorption of oligopeptides from the small intestine might play a significant part in protein absorption (Asatoor *et al.*, 1970a, 1970b). We have therefore studied amino-acid absorption from the small intestine by a perfusion method, and compared the relative rates of absorption of a dibasic amino-acid when perfused either free or as a dipeptide. We report here the findings in a normal subject and in a patient with cystinuria.

SUBJECTS STUDIED AND PROCEDURE

Two subjects were admitted to a metabolic ward for study. One was a normal adult male volunteer, the other a male homozygous cystinuric patient with a long history of renal calculi. This patient has been described previously (Crawhall *et al.*, Case 4). At the time of the investigation he was not being treated with D-penicillamine.

A modified double lumen tube incorporating a proximal balloon was used for the study (Sladen, 1970). Following an overnight fast the subjects were intubated and the tube was allowed to pass until x-ray screening confirmed that the infusion site lay just beyond the duodenojejunal flexure. Perfusion was carried out at a rate of 15 ml. per minute and after a 30-minute equilibration period triplicate samples were collected 30 cm. below the infusion point over a further 30-minute period.

The following solutions were perfused: (1) glycyl-L-lysine 4.2 mmol, (2) glycine 4.2 mmol, (3) lysine hydrochloride 4.2 mmol, (4) glycine 4.2 mmol + lysine hydrochloride 4.2 mmol. All solutions were made isotonic by the addition of sodium chloride and contained polyethylene glycol in a concentration of 4 g. per litre as a non-absorbable marker. The pH was adjusted to 7. Solute absorption was calculated by the generally used formula:

$$A = \left(C_p - C_d \times \frac{P_p}{P_d} \right) \times R$$

Where A is the amino-acid absorption in $\mu\text{mol}/\text{minute}$ per 30-cm. segment; C_p is the concentration of amino-acid in the perfusion solution in $\mu\text{mol}/\text{ml}$; C_d is the concentration of amino-acid in the intestinal aspirate in $\mu\text{mol}/\text{ml}$; P_p is the concentration of polyethylene glycol in the perfusion solution; P_d is the concentration of polyethylene glycol in the intestinal aspirate; and R is the perfusion rate in ml. per minute (Holdsworth and Dawson, 1965).

Free amino-acids and dipeptides were measured chromatographically with a Technicon amino-acid analyser, according to the method of Spackman *et al.* (1958). Polyethylene glycol was measured by a turbidimetric method (Hydén, 1955).

RESULTS

The results are shown in the Chart. In the cystinuric patient lysine was very poorly absorbed, but glycine was absorbed normally. Absorption of both amino-acids was similar when perfused either separately or as a mixture.

Absorption of glycine and lysine from the lumen when perfused at a concentration of 4.2 mmol, either separately, as a mixture, or as the dipeptide glycyl-L-lysine.

When equimolar amounts of the amino-acids were perfused as the dipeptide, absorption of lysine was almost as great in the cystinuric patient as in the normal subject. In the normal subject lysine absorption was also greater from the dipeptide than from the mixture, but the difference was much less striking. Glycine absorption was similar whether perfused as dipeptide or as amino-acid mixture.

During dipeptide perfusion less than 4% of the dipeptide glycine content was recovered as free glycine, and the amount was similar in the two subjects. In contrast, though only 3% of free lysine was recovered in the normal subject, 21% of free lysine was recovered from the lumen of the cystinuric subject. This accounts entirely for the small difference in lysine absorption from dipeptide in the two subjects (see Chart), as disappearance of dipeptide from the lumen was in fact greater in the cystinuric subject (75%) than in the normal one (64%).

DISCUSSION

The demonstration by direct measurement that lysine is very poorly absorbed by the small intestine of a cystinuric patient confirms previous work which has indicated that in cystinuria the active transport system for dibasic amino-acids is defective (Milne *et al.*, 1961).

In cystinuria there is no clinical evidence of malnutrition despite this defect of intestinal transport, and despite the considerable renal loss of dibasic amino-acids and cystine. An alternative mechanism of absorption, perhaps by diffusion, would therefore appear to be likely. Plasma amino-acid levels after oral tolerance tests do indicate that some absorption of free lysine can occur (Hellier *et al.*, unpublished observations), but this method allows no assessment of its extent. The direct measurements reported in the present paper show that lysine absorption is in fact considerably greater when taken as a dipeptide, and it is very likely that in the cystinuric subject most dibasic amino-acid is absorbed in this way.

The extent to which amino-acids are absorbed as free amino-acids, after complete intraluminal hydrolysis of protein, or as oligopeptides after incomplete hydrolysis, has been a disputed question for many years. Recently, however, evidence has been accumulating that not only are dipeptides absorbed both in animals and in man, but that their absorption may be of nutritional significance (Craft *et al.*, 1968; Matthews *et al.*, 1968, 1969).

The inherited diseases in which defects of intestinal transport of specific amino-acids occur provide a unique opportunity for studying the mechanism of dipeptide absorption. Dipeptide absorption has not previously been studied in cystinuria, but Asatoor *et al.* (1970a, 1970b) carried out oral tolerance tests in two patients with Hartnup disease, a very rare condition in which clinically detectable malnutrition occurs due to defective small-intestinal transport of neutral amino-acids. They were able to show convincingly that while there was little or no absorption of neutral amino-acids when the free amino-acid was administered, various dipeptides containing the same amino-acids were readily absorbed. Our findings support their conclusion that in normal subjects amino-acids are absorbed by a dual mechanism either as free amino-acids or as dipeptides.

Free dibasic amino-acids are absorbed very slowly from the human jejunum, and as in animals very low transport constants (K_t and V_{max}) are found (Hellier *et al.*, unpublished observations). In the one normal subject studied lysine was better absorbed as the dipeptide than as free-amino acid, and it is quite possible that absorption of dibasic amino-acids as dipeptides might be of nutritional significance in normal sub-

jects. In animals a very limited number of dipeptides have now been studied, but it would appear that the relative rate of absorption as free amino-acid or as dipeptide differs considerably for different dipeptides (Asatoor *et al.*, 1970b). The same is probably true in man, as Asatoor *et al.* (1970b) showed apparently better absorption of phenylalanine as free amino-acid than as dipeptide, a finding in contrast with our observations on lysine, and Matthews *et al.* (1968) showed diglycine to be absorbed more rapidly than glycine.

The site of hydrolysis of oligopeptides into the constituent amino-acids is unknown, though as only free amino-acids are found in portal vein blood it is reasonable to assume that the peptidases lie somewhere in the mucosal cell. It is of interest that during the dipeptide perfusion in the normal subject only very small quantities of glycine and lysine were detected in the recovered perfusate. In the cystinuric subject, though minimal quantities of free glycine were detected, lysine was found in higher concentrations. The source of this lysine is uncertain. It may have resulted from intraluminal hydrolysis of the dipeptide, free glycine being readily absorbed while lysine accumulated within the lumen. Alternatively, free lysine may have diffused back into the intestinal lumen after dipeptide absorption and intracellular hydrolysis.

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Medical Memoranda

Irradiation-induced Constrictive Pericarditis in Intestinal Lymphangiectasis

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The following case report confirms earlier reports (Peterson and Hastrup, 1963; Wilkinson *et al.*, 1965) that the features of lymphangiectasis with abnormal intestinal protein leak may disappear after pericardiectomy. Constrictive pericarditis in the present case was irradiation-induced; this has never previously been reported in association with intestinal lymphangiectasis.

CASE REPORT

The patient, a 40-year-old woman, had a scirrhus carcinoma locally excised from her left breast in 1959. Subsequently she received a course of radiotherapy. At no time has there been evidence of local recurrence or metastases. Within a year of treatment unusually severe radiation stigmata developed with considerable atrophy of the skin of the left chest, moderate lymphoedema of the left arm, and radiation fibrosis in the upper lobe of the left lung.

She remained well until 1964 when, after a mild chest infection, generalized oedema developed. Initially this responded to diuretics but recurred intermittently until 1966. Since then she has never been completely free from oedema.

Full investigation was refused until January 1967, when persis-