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Intestinal perfusion studies in tropical sprue

2 Movement of water and electrolytes

M. D. HELLIER[1], PREMA BHAT, J. ALBERT, AND S. J. BAKER

From The Wellcome Research Unit, Christian Medical College Hospital, Vellore, Tamil Nadc, India, and St. Thomas' Hospital, London

SUMMARY Jejunal absorption of sodium and water has been investigated in 10 normal Indians and 11 patients with chronic tropical sprue. Normal saline and solutions containing amino acids and dipeptides were studied. In both groups little absoprtion of sodium and water from normal saline was seen. Mean water and sodium absorption from the free amino acid or dipeptide solutions showed no significant difference between the groups and was similar to absorption from normal saline. These results differ from data obtained in normal English subjects where at these concentrations significant stimulation of sodium and water absorption was seen. In a proportion of subjects in both groups net secretion was observed. However, no correlation was seen between mucosal histology or luminal bacteriology and sodium or water movement. These findings in patients with chronic tropical sprue are similar to findings in normal Indians and suggest that jejunal handling of sodium and water is abnormal when compared with normal English subjects, but that the mucosa is not in a secretory phase as seen in certain other diarrhoeal states or in the acute early phase of sprue.

Diarrhoea is usual though not invariable in patients with tropical sprue. In patients in Southern India a large volume watery diarrhoea (>1500 ml/24 h) seems to be particularly common at the onset of the disease and may also occur intermittently as acute episodes during the course of chronic disease, but more commonly there is a moderate increase in stool volume (300-1000 ml/24 h) and the stools are mushy in consistency.

Diarrhoea commonly implies malabsorption of water and electrolytes. However, few quantitative studies have been performed in patients with tropical sprue to investigate water and electrolyte absorption. Two reports have suggested that jejunal secretion of water and electrolytes is a frequent feature of the untreated disease and that this may be rapidly reversible on treatment with antibiotics (Banwell *et al.*, 1970; Corcino *et al.*, 1973). However, patients with large volume watery diarrhoea were chosen in these studies.

The majority of patients with tropical sprue under the care of this unit have chronic malabsorption with moderate volume diarrhoea. The aim of the present study has been to investigate small intestinal water and electrolyte movement in these patients using a perfusion technique.

[1]Address for correspondence: Dr M. D. Hellier, St. Thomas' Hospital, London S.E.1

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Methods

Studies were performed in 10 normal Indians and 11 patients with tropical sprue, described in a previous report (Hellier *et al.*, 1976b). Both groups of subjects were chosen from the same village area. The diets in the two groups were comparable, seven of the 10 normal subjects and eight of the 11 sprue patients being vegetarians. In the sprue patients the diets were considered to be poor or very poor in four, good in two, and fair in the remainder. In the normal subjects diets were poor in two, good in three, and fair in the remainder. The mean weights in the normal and sprue group were 43·6 kg (range 35-60·2 kg) and 38·4 kg (range 33·3-50·4 kg) respectively. Serum albumin levels were low in three of the sprue patients but in none of the normal subjects. The mean stool volume in the sprue patients was 764 ± 262 ml and in the normal subjects 580 ± 175 ml. The difference in mean stool volume was significant ($P < 0.025$). None of the subjects studied had intestinal parasites or evidence of other infections. The normal Indians all had normal vitamin B_{12} absorption and faecal fat excretion and all but three had normal xylose absorption. All patients with tropical sprue had chronic disease (of more than six months' duration), and were not suffering from high volume watery diarrhoea at the time of the study. All had abnormal results for xylose and

vitamin B_{12} absorption tests, repeated on several occasions and steatorrhoea. Jejunal biopsies were taken in all subjects within 48 hours of the study. By western criteria none of these biopsies was normal, all showing some degree of broadening and shortening of the villi, with variable degrees of inflammatory cell infiltrate (Baker and Mathan, 1972). There was no clear cut difference between the two groups, although the changes tended to be rather more marked in the sprue patients.

Perfusions were carried out using a modified double lumen tube, described in detail previously (Hellier *et al.*, 1970).

Measurements of water and electrolyte absorption were made from 0·15 M saline and saline solutions containing the following free amino acids or dipeptides: (1) glycine 20 mmol/l, (2) glycyl-glycine 10 mmol/l, (3) glycine 10 mmol/l + leucine 10 mmol/l, and (4) glycyl-L-leucine 10 mmol/l. These solutions were chosen in order to compare the effect of dipeptides with equivalent amounts of their constituent amino acids, on water and electrolyte absorption. Polyethylene glycol 4000 (4 mg/ml) was added to each solution to act as a nonabsorbable marker.

Amino acids and dipeptides were measured using a paper chromatographic technique described previously (Hellier *et al.*, 1976b). PEG was determined by the method of Hydén (1955).

In six control subjects and six patients with tropical sprue, quantitative bacteriological studies were performed on samples of jejunal juice taken immediately before perfusion through a sterile tube attached to the perfusion tube. Aspirates were transported immediately to the laboratory and cultured for aerobic, anaerobic, and microaerophilic organisms as detailed previously (Bhat *et al.*, 1972). Surface viable counts were made by the spreading method (Cruickshank, 1965) and the results expressed as the number of viable organisms per millilitre of intestinal juice.

Results

ABSORPTION FROM SALINE SOLUTION

In both groups the mean results showed limited absorption of water and sodium. (Fig. 1). In the sprue and normal groups the range for sodium was −3·1 to +14·8 and +2·3 to +12·1 mmol and for water −42 to +52 and −37 to +42 ml respectively. Chloride movement paralleled that of sodium and is therefore not considered separately. In the normal group two subjects secreted water, while in the tropical sprue group two secreted water and one secreted sodium. There was thus no significant difference between the two groups.

ABSORPTION FROM AMINO ACID OR DIPEPTIDE SOLUTIONS

Mean water and sodium absorption from solutions containing either free amino acids or dipeptides showed no significant differences between the two groups and were very similar to absorption from normal saline (Figs. 1, 2a and b). At the concentrations studied there was no evidence for the stimulation of sodium and water absorption by the actively transported non-electrolytes. As in the perfusions with saline, there was a wide scatter of results in both groups, secretion of water and sodium occurring in approximately one-third of subjects in both groups for all solutions. For sodium, the ranges in the sprue and normal groups were: glycine, −8·5 to +25·4 and −7·9 to +11·4; glycyl-glycine −7·9 to +12·1 and −27·8 to +13·3; glycine + leucine, −5·6 to +20·6 and −3·8 to +12·5; glycyl-l-leucine, −12·8 to +16·8 and −3·3 to +10·7 mmol/h respectively. For water, the corresponding ranges were: glycine −54 to +123 and −36 to +53; glycyl-glycine, −68 to +90 and −254 to +66; glycine + leucine, −48 to + 113 and −39 to +59; glycyl-l-leucine,

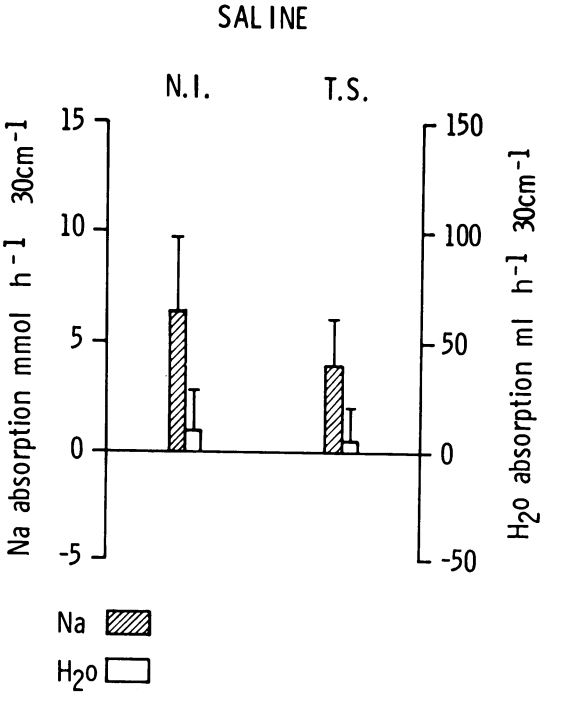

Fig. 1 *Absorption of sodium and water from normal saline in normal Indians (NI) and patients with tropical sprue (TS). Mean results ±1 SD are given.*

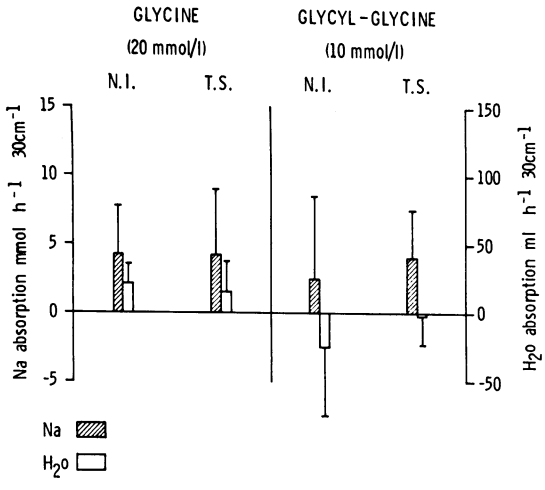

Fig. 2a *Absorption of sodium and water from glycine 20 mmol/l and glycyl-glycine 10 mmol/l solutions in normal Indians (NI) and patients with tropical sprue (TS). Mean results ±1 SD are given.*

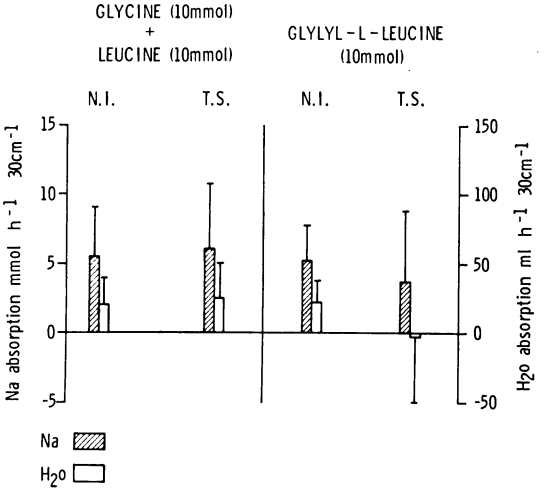

Fig. 2b *Absorption of sodium and water from glycine 10 mmol/l plus leucine 10 mmol/l, and glycyl-L-leucine 10 mmol/l solutions in normal Indians (NI) and patients with tropical sprue (TS). Mean results ± 1 SD are given.*

−156 to +101 and −22 to +58 ml/h respectively.

This contrasts with normal English subjects where stimulation of sodium and water absorption was seen. Thus absorption from glycine (20 mmol/l) and glycyl-glycine (10 mmol/l) solutions was 10·9 ± 4·6 and 13·5 ± 5·8 mmol/l; while water absorption from the same solutions was 81 ± 39·7 and 114 ± 54·4 ml/h respectively. These results were significantly greater ($P < 0.05$) than the results in the Indian subjects in all cases except for sodium absorption, from the glycine solution (Hellier *et al.*, 1976a).

BACTERIOLOGY

The 12 subjects studied included all those who showed secretion of sodium and water. Two of the control subjects and one of the patients with sprue had no viable organisms in the jejunal juice. In the others counts ranged from 10^2 to 10^6 organisms per ml of juice, there being no quantitative difference between the two groups. Further, there was no correlation between the occurrence of water secretion and the luminal flora. Thus net secretion was seen in a control with no viable organisms and net absorption occurred in a control with $2·3 \times 10^6$ coliforms per ml of jejunal juice.

HISTOLOGY

There was no correlation between the histological appearance of the jejunal biopsies and the movement of water or electrolytes.

Discussion

This study has shown that there was no difference in the handling of water and electrolytes in the upper jejunum of normal control subjects as compared with patients with chronic tropical sprue—both groups showed mean net absorption.

There are two previous studies of water and electrolyte absorption in tropical sprue. Banwell *et al.* (1970) in Calcutta studied 13 patients with tropical sprue and showed that, when perfused with a solution of saline and bicarbonate, water and sodium secretion occurred in half, whereas all the control subjects showed net absorption. Corcino *et al.* (1973) studied 10 Puerto Rican subjects with tropical sprue perfused with normal saline. All patients showed net secretion of sodium and water whereas eight controls all showed net absorption.

The reason for the marked difference between the results of the present investigation and those of the above two studies may well reside in the choice of the controls and of the case material in the different studies.

The patients with tropical sprue in the present study were all subjects with established chronic tropical sprue who did not have watery diarrhoea at the time of the study. The subjects of Banwell *et al.* are not well defined and those of Corcino *et al.* are described as having 'active diarrhoea at the time of perfusion'. The impression is that both these groups of investigators were looking at subjects with large volume diarrhoea who might well be expected to show net fluid secretion.

Fourteen out of the 15 patients with 'sprue' studied by Banwell *et al.* (1970) had coliforms in the

upper small bowel and were significantly different from the controls. In the subjects studied by Corcino *et al.* (1973) it is also implied that coliforms were present in the upper small intestine of the patients with sprue. This fact and the response to antibiotics suggest that luminal bacterial infection played a much more significant role in these subjects than in ours, and may account for the observed differences in water movement.

In order further to investigate mucosal water and electrolyte handling the stimulatory effect of actively transported solutes was studied. In normal subjects amino acids and dipeptides stimulate sodium and water absorption (Hellier *et al.*, 1973). By contrast, in certain pathological situations—for example, cholera, coliform infections, and some patients with coeliac disease—this interaction appears to be impaired with secretion of electrolytes occurring in the face of amino acid or dipeptide absorption.

In the presence of either amino acids or dipeptides no increase in absorption of sodium or water was seen in the control subjects or in the patients with sprue over and above that seen from normal saline (Figs. 1, 2a and b). This contrasts with normal English subjects where, with the same solutions, a significant stimulus to absorption of sodium and water was seen (Hellier *et al.*, 1973). To a certain extent the reduced sodium and water absorption can be explained on the basis of reduced amino acid and dipeptide uptake seen in these groups. However, even when absorption of sodium and water was expressed per mole of amino acid or dipeptide absorbed, uptake was still abnormal (Table). This suggests that, at least for the concentrations studied here, the normal interaction between electrolytes and non-electrolytes was impaired both in the normal Indian subjects and patients with tropical sprue.

Mucosal function with respect to amino acid and dipeptide absorption seems to be significantly deranged in apparently normal Indian subjects (Hellier *et al.*, 1976a). It appears that this functional impairment applies also to electrolyte and water handling by the mucosa.

In tropical sprue mucosal electrolyte handling is abnormal when judged by western standards. However, by comparison with apparently healthy Indians selected from a similar socioeconomic background, patients with chronic tropical sprue fall within the range seen in this 'normal' group. Although the sprue patients were not secreting water into the jejunum their mean stool volume was significantly higher than that of the controls. There must, therefore, have been a difference in the handling of water, either in the lower small bowel or in the large intestine.

Table *Sodium and water absorption from solutions of glycine and glycyl-glycine in normal Indians (NI), patients with tropical sprue (TS) and normal English subjects (NE)*

Subjects	*Solutions*					
	Glycine (20 mmol/l)			*Glycyl-glycine (10 mmol/l)*		
	NI	*TS*	*NE*	*NI*	*TS*	*NE*
Na absorption (mmol per mmol glycine or glycyl-glycine absorbed)	0·65 ±0·4	0·52 ±0·59	1·25 ±0·22	0·52 ±1·06	0·62 ±0·84	2·1 ±0·42
	NS	NS		NS	NS	
	NS			NS		
H_2O absorption (ml. per mmol glycine or glycyl-glycine absorbed)	3·2 ±1·81	2·0 ±3·69	9·0 ±1·73	−4·5 ±8·4	−3·8 ±5·3	18·3 ±4·1
	NS	NS		NS	$P < 0.005$	
	$P < 0.025$			$P < 0.05$		

Absorption is expressed as a ratio in mmol Na or ml of water per mmol glycine or glycyl-glycine absorbed. Mean results ± SE are given.

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