Mechanism of malabsorption in giardiasis: a study of bacterial flora and bile salt deconjugation in upper jejunum

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SUMMARY Sixty-three unselected cases of giardiasis, with no evidence of other systemic disease, were screened for evidence of steatorrhoea. No patient had any evidence of protein-energy malnutrition. Seventeen (27 %) of the cases had steatorrhoea; three (17.8 %) of the 17 patients having steatorrhoea also had D-xylose malabsorption. Vitamin B₁₂ absorption was normal in all. Bacterial culture and qualitative analysis of bile salt in jejunal fluid was carried out in all the 17 cases having steatorrhoea as well as 13 cases with normal absorptive parameters (eight cases of irritable bowel syndrome and five cases of giardia infection) who served as controls. Significant bacterial overgrowth was noted in eight of the 17 cases and in none of the 13 controls. All the patients showing bacterial overgrowth had free bile acids in their duodenal aspirate. Free bile acids could also be detected in jejunal aspirates of five of the seven patients having no bacterial overgrowth. Two control cases of giardia infection with normal small bowel function and sterile duodenal aspirate showed evidence of bile salt deconjugation. The significance of these findings is discussed in relation to the pathogenesis of steatorrhoea in patients with giardiasis. The possible role of giardia in bile salt deconjugation is suggested.

Giardiasis is an important cause of malabsorption both in children (Véghelyi, 1939; Cortner, 1961; Nair, 1970) and adults (Manson-Bhar, 1943; Amini, 1963; Antia *et al.*, 1966; Tewari and Tandon, 1974). The mechanism of malabsorption in these patients has not been adequately investigated. The following pathogenic factors have been suggested: (1) a mechanical barrier to absorption (Véghelyi, 1940; Tandon *et al.*, 1974); (2) injury to the intestinal mucosa without invasion (Yardley *et al.*, 1964; Hoskins *et al.*, 1967); (3) mucosal invasion by parasites (Brandborg *et al.*, 1967); and (4) bacterial overgrowth in the upper small bowel (Yardley *et al.*, 1964). A number of investigators have studied the injury to the intestinal mucosa in patients with *Giardia lamblia* infection (Zamcheck *et al.*, 1963; Yardley *et al.*, 1964; Tewari and Tandon, 1974) but we found no published data on bacterial flora of the

upper small gut in these patients. Bacterial overgrowth is known to be associated with deconjugation of bile salts in the stagnant loop syndrome (Gorbach and Tabaqchali, 1969). The present study was therefore carried out to investigate the bacterial content and the bile acid profile in the duodenal aspirate of patients with giardiasis and steatorrhoea.

Methods

Sixty-three patients were selected on the basis of the presence of *Giardia lamblia* cysts in their faecal samples. They were screened for evidence of steatorrhoea by seven day fat balance study (King and Wootton, 1964). Patients whose faecal fat excretion was more than 6 g in 24 hours were investigated by the following laboratory tests: haemogram, serum proteins, barium meal examination of the upper gastrointestinal tract, D-xylose absorption (Santini *et al.*, 1961), Schilling test (Schilling, 1953), and blood immunoglobulins (Fahey and McKelvy, 1965). Eight patients with the irritable bowel syndrome and five patients with giardiasis but with normal small

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bowel absorptive function served as the controls. Bacterial culture and bile studies were undertaken in all the patients with steatorrhoea and in the controls.

Duodenal fluid was obtained after an overnight fast by using a double lumen tube positioned fluoroscopically at the ligament of Treitz. Aspirated fluid was immediately examined for *Giardia lamblia*.

The specimens for bacteriological examination were collected with a sterile syringe in a sterile screw-capped glass container. In addition, a known amount of fluid was immediately inoculated into Robertson's cooked meat media in proportion of 1:10 to recover fastidious anaerobic bacteria. Robertson's cooked meat media was deaeriated before inoculation, and was sealed with sterile liquid paraffin after inoculation. The specimens were transferred to the laboratory as soon as possible, where 10-fold dilutions of the fluid were made in peptone water for aerobic culture from 10^{-1} to 10^{-4} and 0.01 ml of each dilution was inoculated without delay (within one hour of collection) on one blood agar plate, one MacConkey's agar, and one desoxycholate citrate agar plate. Five percent sheep blood agar plates were used in the present study. The plates were incubated aerobically, at 37°C for 48 hours. The contents of the inoculated Robertson's cooked meat medium were also serially diluted in the same way as described above, except that the diluent fluid was deoxygenated charcoal water instead of peptone water. One hundred millilitres of the dilutions were inoculated on blood agar plates. Inoculated blood agar plates were incubated anaerobically in an anerobic jar fitted with a cold catalyst (Torbal Model-AJ-2, Torsion Balance Co. Clifton, New Jersey, USA) for 48 hours. Carbon dioxide could not be added to the anaerobic jar. Only on rare occasions were the uninoculated materials kept in the refrigerator for two to four hours before processing for aerobic culture and the inoculated Robertson's cooked medium was kept in the incubator at 37°C before processing. The number of colonies growing on blood agar plates inoculated with different dilutions of the fluid after 48 hours was recorded and the mean number of colonies grown aerobically and anaerobically per millilitre was then calculated. The dilutions giving less than 10 colonies or more than 200 colonies were disregarded in calculating the mean number. The organisms were then identified on the basis of Gram's staining, motility, cultural characteristics, and biochemical reactions according to Cruickshank (1965), Stokes (1968), and Lennette *et al.* (1970).

Proteins were immediately precipitated from the duodenal aspirates by repeated alcoholic extractions and extraneous lipids were removed by partitioning with n-heptane. These processed samples were stored at -20°C until 24 to 72 hours when they were

streaked on thin layer chromatographic plates. Thin layer chromatography of bile salts was done using plates coated with 250 μ thick layer of Silica gel G (E. Merck) and a solvent system consisting of n-heptane 2:isopropyl ether 1:isopropyl alcohol 1:glacial acetic acid 1 (Kapadia *et al.*, 1971).

Results

Seventeen of the 63 patients showed evidence of steatorrhoea. The clinical features of patients with and without steatorrhoea were compared. Abdominal discomfort or pain was the commonest symptom in both the groups. Recurrent loose motions, flatulence, malaise, and mucus in the stools were the other prominent symptoms. Twenty-four per cent of the patients in the group with steatorrhoea complained of passage of bulky, frothy stools but none of the patients in the other group had this symptom. Weight loss, documented or undocumented, was present in both the groups, but it was more frequent in the patients with steatorrhoea. The duration of diarrhoeal symptoms varied from two months to 12 years. More than half of the patients had had them for more than one year. None of the patients showed any clinical evidence of vitamin deficiencies. Haematological investigations in 17 patients with giardiasis and steatorrhoea revealed a mean serum protein and albumin level of 6.8 g and 4 g % respectively. Mild hypoalbuminaemia (3.26-3.75 g %) was recorded in two. The ESR was raised in four patients. Differential leucocyte count showed an eosinophil count between 5 to 15% in seven and 5% or less in the rest of the cases. Mild anaemia with a haemoglobin value between 10 to 12.9 g % was recorded in eight patients. Blood immunoglobulins were normal in all. Abnormal D-xylose excretion was observed in three of the 17 (17.8%) cases. The Schilling test was normal in all.

Table 1 Results of bacterial culture study in jejunal aspirate from patients with giardiasis and controls

Subjects	Total cases	Cases with:		
		Bacterial overgrowth 10^8 col/ml or more	Minimal growth 10^8 col/ml or less	Sterile culture
Patients with giardiasis with steatorrhoea	17	8 (48%)	1	7
Controls:				
1. Irritable bowel syndrome	8	0	2	6
2. Giardia infection without malabsorption	5	0	0	5

The incidence of bacterial overgrowth (10^5 orgs/ml juice) is shown in Table 1. Eight patients (48%) had an abnormal overgrowth of bacteria, one patient yielded *Nisseria* and micrococci (as both were non-pathogenic organisms they were not considered as an abnormal overgrowth), one patient had a minimal bacterial growth (10^4 org/ml juice), and seven samples were sterile. Two of the eight patients with the irritable bowel syndrome had minimal growth and six had a sterile culture. All the five patients with giardiasis without malabsorption had sterile duodenal aspirate. All the three samples which had to be kept in refrigeration for two to four hours were sterile: two were from patients with giardiasis and steatorrhoea and the remaining one was from a control subject. The types of organism grown and their concentration is shown in Table 2. The organisms were a mixed group of cocci and bacilli, with the gram negative group predominating.

Table 3 shows the incidence of bile salt deconjugation in patients and controls. Fourteen or 82.5% of the patients showed free bile acids in jejunal fluid. None of the eight patients with the irritable bowel syndrome showed deconjugation. Two of the five patients with giardiasis without the malabsorption syndrome showed bile salt deconjugation. The relation between bile salt deconjugation and bacterial overgrowth is shown in Table 4. Of the eight patients

Table 2 Bacteriological data of patients having bacterial overgrowth

Slide no.	Type of organism	Log counts/ml 10
1.	<i>Klebsiella</i>	6.08
	<i>Pseudomonas</i>	5.00
	<i>Coliform</i>	4.90
2.	Anaerobic spore bearers	5.67 (BA) 5.00 (MAC)
3.	<i>Mima Herrellia</i>	5.00
	<i>Staph. albus</i>	4.30
	Micrococci	4.23
4.	<i>Klebsiella</i>	5.90
5.	<i>Klebsiella</i>	5.78
	<i>Strep. viridans</i>	5.30
	<i>Mima Herrellia</i>	5.30
6.	Entero-cocci	6.00
	Micrococci	3.48
7.	Aerobic spore bearer	4.85
	Yeast cells	5.30
	Anaerobic spore bearing bacilli	4.78
8.	<i>Coliform</i>	5.78
	<i>Mima polymorpha</i>	5.78
	Anaerobic spore bearer	4.85
	<i>Staph. albus</i>	5.48

BA: blood agar media.

MAC: MacConkey media.

Table 3 Bile salt deconjugation in patients and controls

	Total no. of cases	Cases showing deconjugation (no.)	(%)
Patients	17	14	82.5
Controls:			
1. Irritable bowel syndrome	8	0	0
2. Giardiasis without malabsorption	5	2	40.0

Table 4 Relation between bacterial overgrowth and bile salt deconjugation in giardiasis patients with steatorrhoea and control subjects

Bacterial culture of jejunal aspirate	Total cases	Cases showing free bile acids
Patients with:		
Significant bacterial overgrowth	8	8
'Insignificant' growth	1	1
No bacterial growth	7	5
Controls with:		
Insignificant bacterial growth	2	0
No bacterial growth	6	0

with significant bacterial overgrowth all showed evidence of deconjugation. One patient with a bacterial count of less than the accepted level of significance also had free bile acids in the jejunal aspirate. Of the remaining seven patients with giardiasis with sterile jejunal aspirates, five had evidence of bile salt deconjugation. Two of the five controls who had giardia infection and normal small bowel function showed the presence of free bile acids in the jejunal aspirate even though the duodenal aspirate was sterile. Vegetative form giardia were demonstrated in fresh jejunal aspirates in all the patients examined.

Steatorrhoea was recorded in 27% of patients with giardiasis in the present study. Reports in the literature are very variable and fat malabsorption has been reported to range from zero (Palumbo *et al.*, 1962; Kotcher *et al.*, 1966) to 50% (Tewari and Tandon, 1974) of the patients with *Giardia lamblia* infestation. Either a variable load of infection or certain unidentified host factors may be responsible for the discrepancies in the results of the fat malabsorption in these patients. Undoubtedly giardia infection gives rise to mild to moderate steatorrhoea. The severe degree of fat malabsorption that has been reported in other diffuse small bowel disorders (Bossak *et al.*, 1957) is not observed in this disease. However, if one considers a high prevalence of giardia infection—for example, to the extent of 10% of the population in developing countries where the people are already receiving inadequate nutrients in their diet—even mild to moderate steatorrhoea is likely to become an important contributory factor

for protein-energy malnutrition. (Tewari and Tandon, 1974).

The clinical features of patients with and without steatorrhoea were almost the same as those described in the literature. However, patients with steatorrhoea seem to have a more frequent history of passage of large bulky stools associated with weight loss when compared with the group with giardiasis but without steatorrhoea. It is felt that steatorrhoea in the present series of patients was aetiologically related to *Giardia lamblia* infestation and it seems unlikely that the patients belong to any other category of intestinal disease giving rise to malabsorption. None of the patients had the clinical or biochemical features of dietary protein-energy malnutrition that would raise the possibility of hypoproteinaemic enteropathy. Tropical sprue also seems to be an unlikely possibility, as Schilling's test was normal in all the patients and D-xylose excretion was abnormal only in a small number of three patients and none was found to be anaemic. The reversibility studies (Véghelyi, 1939, 1940; Katsampes *et al.*, 1944; Powell, 1956; Tewari and Tandon, 1974) on malabsorption in patients with giardiasis, after eradication of the parasite, support the possibility of a direct aetiological relationship between *Giardia lamblia* and malabsorption.

The results of the present study suggest that the bacterial overgrowth and bile salt deconjugation are the possible causes of fat malabsorption in patients with giardia infestation. The bile salt deconjugation was recorded in 14 of the 17 patients and bacterial growth with significant colony counts was observed in 48% of them. None of the eight control cases of irritable bowel syndrome had either bile salt deconjugation or significant bacterial overgrowth in their jejunal aspirates. However, two of the controls with giardia infection and normal small bowel function had bile salt deconjugation without any bacterial overgrowth in the duodenal aspirate.

The mechanism of steatorrhoea in patients with bacterial colonisation of the small intestines has been the subject of study for many years. Normal morphology of the absorptive cell and villi by light (Rubin and Dobbins, 1965) and electron microscope (Tabaqchali *et al.*, 1968) does not support the possibility of direct invasion of mucosa by bacteria. Some investigators (Neale, 1967; Tabaqchali and Booth, 1970) have suggested that protein deficiency may result because of bacterial degradation of dietary protein in the gastrointestinal tract, and hence there is a theoretical possibility of hypoproteinaemic enteropathy in patients with bacterial colonisation of the upper gut. However, normal or only slightly low serum protein values and the absence of clinical features of protein-energy mal-

nutrition rule out such a possibility in the present series of patients. Wirts and Goldstein (1963) suggested the possibility that an impaired lipolysis as a result of bacterial inactivation of lipase was responsible for fat malabsorption. Experimental studies (Donaldson, 1965) have failed to confirm this possibility. Dawson and Isselbacher (1960) were the first to suggest that altered bile salt metabolism by bacteria might be responsible for derangement of fat absorption. Normally, colonic bacteria convert the conjugated bile salts to unconjugated forms in the colon. It was suggested that bacterial flora of the upper gut might do the same. The alteration of normal bile salt metabolism by bacterial deconjugation within the lumen could cause steatorrhoea in two ways. Firstly, the unconjugated bile acids might be toxic to intestinal mucosa and, secondly, deconjugation might result in a reduction of the concentration of conjugated bile salts. Tabaqchali and Booth (1966) showed that patients with high bacterial counts in the jejunal fluid with steatorrhoea had large quantities of unconjugated bile acids in lumen of small intestine. Treatment with broad spectrum antibiotics reduced jejunal bacterial counts and the disappearance of free bile acids, associated with improvement in fat absorption.

It is now generally agreed that, in patients with small bowel bacterial overgrowth, the flora come to resemble faecal flora. An association between the presence of bacteroides and bile salt deconjugation has been stressed in the literature (Tabaqchali, 1974), but none of the patients in the present series had these bacteria in the small bowel. Techniques used for anaerobic culture in this study might have missed the bacteroides or this might be a characteristic finding in our patients that was probably related to the internal milieu of the small bowel. The association of bile salt deconjugation with aerobic as well as anaerobic bacteria in the absence of demonstrable bacteroides in the small bowel has been reported by a few earlier investigators (Goldstein *et al.*, 1969). Hydrolysis of conjugated bile salts has been produced *in vitro* by *Streptococcus lactis* and *Lactobacillus buchneri* (Shimada *et al.*, 1969) as well as by *E. coli* (Norman and Shorb, 1962; Portman *et al.*, 1962; Midtvedt and Norman, 1967; Dickinson *et al.*, 1971). It is thus possible that the aerobic coliforms or the streptococcal organisms isolated in our patients may explain the pathogenesis of bile salt deconjugation even in the absence of bacteroides or other anaerobic micro-organisms.

Bacterial colonisation seems to be primarily responsible for bile salt deconjugation in the patients with giardia infection. However, it is interesting to note that seven patients with giardiasis in the present series (five study and two control group) had free bile

acids in the bacteriologically sterile jejunal aspirates. It is likely that *Giardia lamblia* itself is capable of deconjugating bile salts. Further *in vitro* studies to confirm the direct role of *Giardia lamblia* in bile salt deconjugation are warranted in the light of the present observations.

Two patients with giardia infection with bile salt deconjugation did not have steatorrhoea. Quantitative aspects of bile salt deconjugation need to be studied to explain this finding.

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