Giardiasis: clinical and therapeutic aspects

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SUMMARY Malabsorption was present in 29 of 40 symptomatic patients with giardiasis. Twenty-three had impaired D-xylose absorption; in 20 vitamin B₁₂ absorption was low, and 15 patients had steatorrhoea. More severe malabsorption was associated with more marked histological abnormalities. Metronidazole, 2.0 g as a single daily dose on three successive days, produced a parasitological cure rate of 91%. In contrast, the standard course of mepacrine, 100 mg thrice daily for 10 days, eradicated the parasite in only 63% of patients. Improvements in absorption and jejunal morphology followed anti-giardial treatment. Tetracycline in eight patients failed to eradicate the parasite, intestinal absorption was unaltered, and histological appearances of the jejunal mucosa often deteriorated.

Giardia lamblia, a flagellate protozoan parasite of the human upper small bowel, is commonly associated with diarrhoea in the tropics (Antia *et al.*, 1966; Ingram *et al.*, 1966; Kapoor and Mody, 1968), though symptomatic giardiasis is reported from temperate areas (Moore *et al.*, 1969; Brodsky *et al.*, 1974; Brady and Wolfe, 1974). The most common symptoms reported are abdominal discomfort, abdominal distension, diarrhoea with soft, offensive, yellow stools, and lassitude.

In experimental infections of prison volunteers, Rendtorff (1954) found that spontaneous eradication of the parasite was the rule but clinical experience indicates that patients may be infected and symptomatic for several years (Alp and Hislop, 1969). It is likely that immunological mechanisms may be involved in the process of eradication (Hermans *et al.*, 1966; Ament and Rubin, 1972).

Malabsorption of D-xylose and fat is well documented in giardiasis (Amini 1963; Yardley *et al.*, 1964; Alp and Hislop, 1969; Ament and Rubin, 1972; Tewari and Tandon, 1974). Vitamin B₁₂ malabsorption has been uncommon in previous reports (Antia *et al.*, 1966; Ament and Rubin, 1972; Ament, 1972; Notis, 1972; Tewari and Tandon, 1974). We report here the findings in a consecutive series of patients with giardiasis in whom malabsorption was common and vitamin B₁₂ malabsorption was particularly frequent.

Methods

PATIENTS

Forty adult Caucasians with giardiasis, 26 males and 14 females, were studied. Half were young adults who had acquired the infection during overland travels in Africa, India, or other parts of Asia. This group usually took locally available food and water. Three subjects were infected on brief trips to Lenin-grad, USSR. The remainder were businessmen, academics, or technical assistance personnel who had worked in Africa or Asia (Fig. 1). They usually adhered to a more Western diet and life-style when abroad. These 40 subjects represent a consecutive series of patients with giardiasis initially investigated because they had profuse diarrhoea of undetermined cause or severe diarrhoea and giardiasis or symptoms suggestive of malabsorption. They were investigated at varying intervals after their arrival in Great Britain.

In eliciting histories particular attention was paid to symptoms referable to the gastrointestinal tract. Clinical evidence of vitamin deficiency and malnutrition was noted from the history and physical examination. Evidence of recurrent infections or gastrointestinal symptoms in the past was sought. Sigmoidoscopic examinations were performed in all cases.

Routine haematological and biochemical investigations, including serum protein levels, plasma transaminase and bilirubin levels were performed in all patients. Serum immunoglobulin levels were

Fig. 1 The distribution of geographical regions in which patients were infected. One patient was infected in the Caribbean. M-0 = ○; M-1 = ●; M-2 = X.

measured by radial immunodiffusion. Serum B₁₂ and folate levels were estimated.

Stool samples were examined microscopically after formol-ether concentration (Allen and Ridley, 1970) for cysts, ova, and larvae of parasites. Stools were cultured for bacterial pathogens. Barium follow-through examinations were used to demonstrate anatomical or inflammatory small bowel lesions. ¹⁴C glycocholate breath tests were done as an *in vivo* test for bile salt deconjugation (Fromm and Hofman, 1971).

Patients were investigated in a metabolic ward so that particular attention was paid to obtaining complete collections for absorption tests. Intestinal

absorption was assessed by urinary D-xylose excretion after a 25 g oral load, by Schilling tests using ⁵⁸Co-labelled vitamin B₁₂ with hog intrinsic factor and by faecal fat excretion over a 72 hour period during which patients consumed a diet containing 100 g of fat per day. Stool weights were recorded during this time.

Using a Quinton biopsy instrument or a Watson biopsy capsule, modified by the addition of an aspirating tube, jejunal fluid and jejunal biopsies were obtained under fluoroscopic control from the first loop of the jejunum. Direct microscopy of uncentrifuged, freshly obtained specimens of jejunal aspirate was performed looking for trophozoites or

pre-cysts of *Giardia lamblia*. When the biopsy capsule was retrieved, any flecks of mucus adhering to the capsule or biopsy were mounted in saline under a cover slip for direct microscopy as above (Brady and Wolfe, 1974). The mucosal surface of a portion of the biopsy was smeared on a slide, then covered with saline and a cover slip for examination by direct microscopy (Kamath and Murugasu, 1972). A piece of the biopsy was homogenised in glycerol transport medium and stored at -20°C after rapid freezing for bacteriological studies (Drasar *et al.*, 1969). The remainder of the biopsy was orientated mucosal side uppermost on a flat surface and fixed in a modified Susa fixative. The dissecting microscope appearances of the mucosa were recorded (Booth *et al.*, 1962) noting the observer's assessment of the most numerous form or forms of villi present—that is, finger shaped villi, approximately equal numbers of finger and leaf shaped villi, etc. After paraffin embedding, 5μ sections of the jejunal biopsy were cut and stained with haematoxylin and eosin. Histological appearances were assessed blind by one of us (D.S.R.) and graded according to a predetermined scale (normal or abnormal grades I to V), which is described in detail elsewhere (Ridley and Ridley, 1976). The abnormalities in grade I were epithelial only, while grade V indicated subtotal villous atrophy.

After initial assessment patients were treated with metronidazole 2.0 g as a single dose on three successive days (Khambatta, 1971; Petersen, 1972; Green *et al.*, 1974; Madanagopalan *et al.*, 1975) or mepacrine 100 mg, thrice daily for 10 days, or tetracycline 250 mg four times daily for four weeks. In acute tropical sprue enterobacterial colonisation of the jejunal mucosa has been described and treatment with tetracycline produced elimination of the organisms and improvement in absorption (Tomkins *et al.*, 1975). If a similar state obtained in giardiasis then similar responses to antibiotic therapy might be anticipated. The nature of this treatment was explained to patients and their consent for it obtained. Patients who received metronidazole were advised to avoid alcohol for the duration of treatment because of its reported disulfiram-like actions (Taylor, 1964). At reassessment one to three months after treatment abnormal absorption tests were repeated, parasitological examinations were repeated, and further jejunal biopsies were obtained. If parasites were still present further treatment was given with mepacrine or metronidazole and follow-up continued.

For comparison, we include results of similar investigations from a group designated 'overland controls' (OC) who did not have malabsorption and a group of patients with untreated acute

tropical sprue (TS). These groups have been described in detail elsewhere (Tomkins *et al.*, 1974; Tomkins *et al.*, 1975). Student's *t* test was used for statistical comparison of results.

Results

G. lamblia was the only parasitic pathogen found in these patients and no bacterial pathogens were isolated by stool culture. The parasitological diagnosis was made on stool examination in 34 patients (85% of the whole group). Cysts were not found in all stools examined and we found that the frequencies of positive stool examinations were similar in the different patient groups irrespective of the presence or absence of malabsorption. Forty-seven per cent of stools from patients with normal absorption contained cysts and 44% of stools from subjects with severe malabsorption contained cysts of the parasite. In six patients (15%) the parasitological diagnosis was made only on examination of mucosal impression smears or jejunal aspirate. Three of these patients had severe diarrhoea and marked malabsorption.

Three groups of patients designated M-0, M-1, and M-2 were defined. Eleven patients comprised M-0 and all had normal intestinal absorption. Ten patients had impaired absorption of a single test substance (M-1) and 19 patients constituted the third group (M-2) who had malabsorption of two or three substances. It is notable that three patients in the M-2 group had acquired the infection in Africa, two were infected in the Middle East, and two were infected in Europe (Leningrad, USSR, and Italy respectively). Figure 1 shows the countries in which subjects were infected. In one subject we were able to estimate the incubation period to be five days at the most before the onset of upper abdominal discomfort, abdominal distension, nausea, and diarrhoea. In the whole group diarrhoea, lassitude, and abdominal distension were the most common symptoms at presentation (Fig. 2). These symptoms were more marked in the M-2 group than in M-0. Weight loss was prominent in the M-2 group. The median duration of symptoms was 17 weeks in M-0 (range four to 104 weeks), 12 weeks in M-1 (range one to 260 weeks), and nine weeks in M-2 (range 12 days to 52 weeks). If M-2 subjects who malabsorbed all three substances are considered, then this value is seven weeks (range 12 days to 16 weeks). A few patients presented within days of their arrival in Britain but 11 of the M-2 category had been here for over a month (Fig. 3).

Despite the frequent occurrence of weight loss, the nutritional status of our patients was good. None had hypoproteinaemia or hypogamma-

Fig. 2 Symptoms at presentation to the Hospital for Tropical Diseases and their frequency in the three groups of patients.

Fig. 3 Interval in weeks between arrival in Britain and presentation at the Hospital for Tropical Diseases for the three groups of patients with giardiasis.

globulinaemia. Selective IgA deficiency was not found in any of those subjects examined. The mean serum folate was only slightly lower (4.6 ng/ml) in the M-2 group than the M-0 group (6.0 ng/ml) but the mean serum vitamin B₁₂ level was significantly lower in M-2 (mean $\pm$ SE = 198 ± 94.5 pg/ml) than in M-0 (301 ± 32.3 pg/ml, $P < 0.05$).

All other haematological and biochemical data were similar in the three groups. Barium follow-through examinations were performed in 35 patients. No evidence of anatomical or chronic inflammatory bowel lesions was found. These studies did show thickened mucosal folds and dilated loops of small bowel in 25 subjects and normal appearances in 10 others. ¹⁴C-glycocholate breath tests were normal in 13 of 14 patients. The single abnormal result was obtained in a patient who had been successfully

treated for an abdominal lymphoma with external radiotherapy four years before presentation to us. In this subject we found no evidence of an anaerobic microflora in the proximal jejunum.

Malabsorption was present in 29 patients. Twenty-three malabsorbed D-xylose. Vitamin B₁₂ absorption was abnormal in 20 patients and 15 had steatorrhoea (Fig. 4 and Table 1). The most severe abnormalities were present in the M-2 group. Mean stool weights were increased in the groups with malabsorption and the mean value in M-2 was significantly higher than in M-0 and M-1 (Table 2). Morphological abnormalities of the jejunal mucosa were common (Fig. 5a, b). In general, more marked histological abnormalities were associated with more marked impairment of intestinal absorption. Significant

Fig. 4 Absorption studies and stool weights in overland control subjects (O.C.), patients with acute tropical sprue (T.S.), and giardiasis patients before treatment. Interrupted lines indicate the lower or upper limit of the normal range for each test as appropriate. $\circ$ = normal Vitamin B₁₂ absorption test; $\blacksquare$ = abnormal Vitamin B₁₂ absorption test (Chanarin 1969).

Table 1 Distribution of abnormal absorption tests in M-2

Test substances malabsorbed			No. of patients
D-xylose	Fat	Vitamin B ₁₂	12
D-xylose	—	Vitamin B ₁₂	5
D-xylose	Fat	—	1
—	Fat	Vitamin B ₁₂	1

Table 2 Mean stool weights in three groups of patients

	Mean stool weight (g/24 h)		
	M-0	M-1	M-2
SEM	147	188	351
Range	12-1	21-0	42-9
	78-197	99-288	113-846

The mean value for M-2 was significantly higher than that for M-0 ($P < 0.005$) and M-1 ($P < 0.02$).

Fig. 5 A. The dominant form or forms of villi on dissecting microscopy of jejunal biopsies at initial assessment: F = finger-shaped villi; L = leaf-shaped villi; R = ridge formations; C = convolutions. B. The histological grading of jejunal biopsies in A.

enterobacterial colonisation of the jejunum was found in M-2 patients. These findings will be reported in detail elsewhere. It is notable that functional and morphological abnormalities in M-2 giardiasis patients were similar to patients with acute untreated tropical sprue (Figs. 4 and 5).

Treatment was not allocated in a randomised way to similar groups of patients, so that differing responses cannot be compared statistically. A single course of metronidazole eradicated *G. lamblia* in 20 out of 22 patients (91%). A second course of metronidazole increased the parasitological cure rate to 95%. Mepacrine eradicated the parasite in five out of eight patients (63%). *G. lamblia* persisted in seven out of eight patients given tetracycline. Intestinal absorption consistently improved in those who took metronidazole or mepacrine and in Table 3 we show those abnormal absorption tests which returned to the normal range. Changes in absorption after tetracycline were minimal in most cases. Histologically, the effect of successful anti-giardial treatment was improvement in grading (Fig. 6) with elimination of cellular infiltrate in the lamina propria and partial repair of the epithelial damage. Sequential biopsies from one patient treated with metronidazole are shown in Figs. 7 and 8.

Table 3 Number of abnormal tests of intestinal absorption that returned to normal range after single course of medication shown

Medication	Test of absorption		
	D-xylose	Schilling test	Faecal fat
Metronidazole	5/9	6/9	5/6
Mepacrine	2/3	3/4	2/2
Tetracycline	0/6	2/4	0/4

Fig. 6 Histological grading of jejunal biopsies before and after treatment.

After tetracycline histological appearances often deteriorated (Fig. 6).

Discussion

Though the pathogenicity of *G. lamblia* in man has been disputed (Palumbo *et al.*, 1962), there is a substantial body of evidence which supports the role of the parasite in causing symptomatic disease in man. Diarrhoea is the most common symptom in giardiasis. Rendtorff (1954) showed in prison volunteers that *G. lamblia* alone produced diarrhoea. Studies from Asia have shown that the parasite is found much more commonly in subjects with diarrhoea than in asymptomatic subjects (Ingram *et al.*, 1966; Antia *et al.*, 1966). Antia *et al.* (1966) showed further that *G. lamblia* was present in only 5.5% of persons with miscellaneous gastrointestinal symptoms or in 6.7% of subjects with dysentery—that is, diarrhoea with blood in the stools, a symptom not noted in

Fig. 7 The pre-treatment biopsy in an M-2 subject (S.O.), showing marked grade II histological abnormality.

Fig. 8 Post-treatment (metronidazole) biopsy in patient S.O. showing improvement to minor grade I histological abnormality.

giardiasis. These figures are very close to the prevalence of 4.4% in asymptomatic carriers. In marked contrast they reported a prevalence of 23.3% in subjects with non-dysenteric diarrhoea. Epidemic giardiasis causing diarrhoea has been reported from Aspen, Colorado (Moore *et al.*, 1969) and in groups of tourists visiting Leningrad, USSR (Brodsky *et al.*, 1974). A persisting bowel upset that continues for weeks or months is typical of giardiasis (Lancet, 1974), but this is not a feature of acute undifferentiated diarrhoea of the tropics. We found no evidence of any other gut pathogen in our patients.

Malabsorption in giardiasis has been reported from a variety of geographical locations (Amini, 1963; Yardley *et al.*, 1964; Antia *et al.*, 1966; Alp and Hislop, 1969; Moore *et al.*, 1969; Tewari and Tandon, 1974). The comparative paucity of reports probably relates to the fact that giardiasis is most common in countries where physicians' time and hospital facilities cannot be expended on the study of an easily treatable gastrointestinal infection. A number of the studies noted above are from areas in which tropical sprue is endemic and it might be suggested that in these studies *G. lamblia* is a non-pathogenic commensal. Just over half our total series of patients were infected in the Indian subcontinent and South-East Asia, but, of seven M-2 subjects, three were infected in Africa, two in the Middle

East, and one each in Italy and Leningrad. Malabsorption associated with giardiasis has a wider geographical distribution than tropical sprue, for this entity has not been encountered in Africa (Cook, 1974). Though there is no specific feature which distinguishes tropical sprue, it usually improves in response to a prolonged course of antibiotics, often with folate or vitamin B₁₂ supplements (Guerra *et al.*, 1965; O'Brien and England, 1971; Rickles *et al.*, 1972). Mepacrine, a commonly used anti-giardial drug, is known to have antibacterial actions *in vitro* (Seligman and Mandel, 1971), but in the short courses used in the treatment of giardiasis (five to 10 days) it seems unlikely to produce the same results as prolonged antibiotic treatment in tropical sprue.

Spontaneous recovery is known to occur in tropical sprue but this usually occurs when subjects receive an adequate diet. Many of our subjects had been in Europe or Britain and taking normal diets for weeks or months before presentation to us, by which time spontaneous resolution of tropical sprue would have occurred. Further giardiasis has been associated with malabsorption in non-immunodeficient subjects who have acquired the infection in temperate climates (Yardley *et al.*, 1964; Hoskins *et al.*, 1967; Morecki and Parker, 1967; Cain *et al.*, 1968; Moore *et al.*, 1969) and in our patients visiting

Italy and Leningrad. Mepacrine in the former subjects eradicated the parasite and absorption improved.

In our patients metronidazole eradicated the parasite and improvement in function and morphology followed. This drug is known to be effective against *Entamoeba histolytica* and *Trichomonas vaginalis*, both pathogenic protozoan parasites, and has anti-bacterial actions on obligate anaerobic bacteria only (Garrod *et al.*, 1973). Though anaerobic bacteria are associated with malabsorption in a number of clinical syndromes (Losowsky *et al.*, 1974), we found no evidence of anaerobic small bowel overgrowth from our bacteriological studies, nor did we find any evidence of bile salt deconjugation as judged by negative ^{14}C -glycocholate breath tests.

The possibility that improvement in absorption after metronidazole is a non-specific effect of the drug should be considered. In a series of eight immunodeficient subjects with malabsorption one subject who did not harbour *G. lamblia* received metronidazole for several weeks without change in absorption. In contrast, seven subjects who had proven giardiasis received metronidazole; the parasite was eradicated in each case, jejunal morphology improved, and absorption returned to normal (Ament and Rubin, 1972). Two subjects had received tetracycline before without effect and one subject had required systemic steroids to keep him alive. After metronidazole his malabsorption regressed, he put on weight and steroids were withdrawn. It is notable that after treatment of giardiasis in these subjects malabsorption regressed despite the presence of an abnormal small bowel flora at follow-up assessment. Morphological improvements were marked in these subjects who had multiple biopsies taken both before and after treatment. From these findings it seems likely that in some patients *G. lamblia* can cause malabsorption. The circumstances in which this occurs are ill understood at present but do not seem to be entirely related to the presence of an abnormal small bowel flora.

We found D-xylose and fat malabsorption to be common in the subjects we studied. The prevalence of vitamin B₁₂ malabsorption was very high (present in 50% of the whole group and 69% of those with malabsorption). H. A. K. Rowland at this hospital (1974, unpublished) found a similar prevalence (67% of 55 patients). These results confirm the earlier but infrequent reports of malabsorption of this vitamin in giardiasis (Antia *et al.*, 1966; Ament and Rubin, 1972; Notis, 1972; Ament, 1972).

The mechanisms of malabsorption in giardiasis remain obscure. The total parasite load may be

important. Disturbance of intestinal motility, luminal competition by the parasite for substrates (Ament, 1972), bacterial colonisation (Yardley and Bayless, 1967; Ament and Rubin, 1972; Tandon *et al.*, 1974) and epithelial damage produced by the parasite (Morecki and Parker, 1967) have been suggested as contributory factors. The role of tissue invasion in producing malabsorption is difficult to assess for the reports of its occurrence are very few (Morecki and Parker, 1967; Brandborg *et al.*, 1967; Brandborg, 1971). Circulating antibody to *G. lamblia* in subjects with malabsorption (Ridley and Ridley, 1976) may indicate either that mucosal invasion is much more common than we at present appreciate or that this is only a marker of increased mucosal permeability with resultant absorption of parasite antigen. *Entamoeba histolytica* is often harboured by asymptomatic individuals. Experimental work has shown that axenically cultured amoebae must be associated with live bacteria for a minimum of 12 hours before invasive amoebiasis can be produced in laboratory animals (Wittner and Rosenbaum 1970). From our bacteriological studies we found evidence of enterobacterial contamination of the jejunum in patients with marked malabsorption, whereas no bacteria were present in subjects malabsorbing only one substance or with normal absorption. The possibility of synergism between enterobacteria and *G. lamblia* to cause damage to the intestinal mucosa cannot be excluded.

In any subject who has a bowel upset that persists for weeks or months after an initial acute onset the possibility of giardiasis should be considered, even in subjects whose travels have been limited to Europe. Single negative stool examinations are insufficient to exclude the diagnosis and small intestinal aspiration and biopsy may be necessary to demonstrate the parasite. The satisfactory therapeutic response to eradication of the *G. lamblia* with improvement in mucosal function and morphology suggests a pathogenic role for this parasite but the mechanisms await elucidation.

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