

SMALL INTESTINAL ABSORPTION STUDIES AND BIOPSY FINDINGS IN ASIANS, IN SINGAPORE*

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In this paper we present the results of studies carried out on 30 male normal controls, and 27 patients admitted to a general medical unit in Singapore, with symptoms indicative of a malabsorption syndrome and a severe anaemia. The investigations and treatment of this group of patients indicate that tropical sprue is not uncommon in the local population, and affects Indians more than the other races living in Singapore. Further, about half of our patients with tropical sprue did not have either diarrhoea or steatorrhoea.

MATERIALS AND METHODS

Subjects

Control Subjects: Studies were carried out on 10 Chinese, 10 Malays and 10 Indians. All were males and had been admitted to the Medical Unit, Thomson Road General Hospital, Singapore, suffering from a variety of non gastrointestinal diseases. All were healthy and had recovered from their original complaints at the time of study. All gave informed consent. Their ages varied from 15 years to 55 years. No studies were carried out in female subjects since all who were approached for this purpose were reluctant to undergo a jejunal biopsy.

The Patients: Since 1966 all patients admitted to the same unit with a megaloblastic anaemia have also been studied. At the beginning of this study it was also intended to investigate non anaemic patients presenting with a combination of a sore tongue, anorexia, dyspepsia, weight loss and diarrhoea as these symptoms are suggestive of an underlying malabsorption syndrome leading to multiple nutritional deficiencies. However, very few patients without a megaloblastic anaemia had a combination of these symptoms and when these patients were investigated it was found that they had normal jejunal biopsies and had no malabsorption. In the past 2 years 37 patients with a megaloblastic anaemia were investigated. 10 patients were

found to be suffering from Addisonian pernicious anaemia and 6 of them have been reported on elsewhere (Jayaratnam *et al*, 1967). The remaining 27 patients were found to be suffering from tropical sprue and are discussed in this paper.

Complete blood counts, marrow smears, serum iron, proteins, calcium, phosphate and blood urea estimations were carried out on the control subjects and patients. In 10 patients serum B12 and folic acid assays were also carried out by Prof. D. L. Mollin at the Royal Postgraduate Medical School, London. Augmented histamine test meals (Kay, 1953) were also carried out on the patients. Stools were examined for ova and cultured and barium meal studies were carried out on all the controls and the patients.

ABSORPTION STUDIES

The ability to absorb dietary fat, Vitamin A, D-xylose and Co⁵⁸ Vitamin B12 was investigated in all the control subjects and most of the patients.

Faecal Fat: A diet containing at least 100 grams of fat, 70 grams being in the form of butter, was administered for a week before estimating the fat content of a 72 hour collection of faeces, by the method of Van de Kamer *et al* (1949).

Vitamin A: 400,000 units of Vitamin A was administered orally to fasting subjects. Blood for Vitamin A estimation was taken just before and 5 hours after the administration of Vitamin A. Food was allowed after the Vitamin A was administered. The Vitamin A content of the blood was estimated by the method of Paterson and Wiggins (1954).

D-xylose: 5 grams of D-xylose was fed to fasting subjects and the quantity of D-xylose excreted in the urine in the next 5 hours was estimated by the method of Roe and Rice (1948).

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Vitamin B12: The absorption of Vitamin B12 was estimated by the method of Schilling (1953) using Co⁵⁸ labelled Vitamin B12 administered simultaneously with hog intrinsic factor.

JEJUNAL BIOPSIES

These were obtained with a Crosby capsule (Crosby and Kugler, 1957) from the first foot of the jejunum. The specimen was stretched flat on gel foam, examined and photographed through a dissecting microscope, and later serially sectioned. Sections were stained with haematoxylin and eosin, and the Gomori trichrome stain (for connective tissue). The mucosal pattern was classified on the lines suggested by Booth *et al* (1962) and Baker *et al* (1962) as consisting of fingers, leaves, ridges, convolutions or as being flat. The serial section showing the tallest and most slender villi was selected and measurements were made with a micrometer of the total mucosal thickness, the height and width of 6 villi and the heights of columnar cells at the tips of the 6 villi. The appearance of the substantia propria and the epithelium was noted as well as the degree of round cell infiltration which was graded as mild, moderate or severe. As suggested by Roy-Choudhury *et al* (1966) we have classified the histological appearance of the mucosa into 4 grades, Grade 0 being normal and Grades 1 to 3 being abnormal (Figs. 1 to 5).

Grade 1 (equivalent to the "mildly abnormal" of Rubin *et al*, 1960). The average height of the villi is between 300 and 350 μ . The epithelium is predominantly columnar in type. There is a minimal increase in chronic inflammatory cells in the substantia propria.

Grade 2 (equivalent to the "moderately abnormal" of Rubin and the "partial villous atrophy" of Doniach and Shiner, 1957). The average height of the villi is between 150 μ and 300 μ . The epithelium is more abnormal, cuboidal and moderately infiltrated with round cells. There is a moderate increase in the infiltration of the substantia propria with plasma cells, lymphocytes and eosinophylls.

Grade 3 (equivalent to the "severely abnormal" of Rubin and the "subtotal villous atrophy" of Doniach and Shiner). (a) There are no true villi—only elevations less than 150 μ in height. (b) a flat surface. The epithelium is extremely abnormal, flattened and irregular in size, shape and staining of the cytoplasm and nuclei. There is a severe infiltration of the

epithelium with round cells. The brush border is missing in many areas. There is a great increase in the round cell infiltration of the substantia propria.

THERAPY

All patients on admission were interviewed by the hospital dietician for details of their home diet and were then placed on a diet closely resembling their home diet. The fat content of the diet was increased to at least 100 grams a day by giving a supplement of 70 grams of butter daily. After all the above investigations except the Schilling test had been carried out, patients were placed on a 10 day therapeutic trial with small parenteral doses of folic acid (0.2 mg. a day) or Vitamin B12 (3 μ g. a day). Most of the patients were given folic acid but 4 patients whose diet had been more or less of a vegetarian nature were given Vitamin B12. During the trial, the haematological response was closely followed up by estimations of the haemoglobin, reticulocyte counts and marrow smears. At the end of the trial patients were placed on full pharmacologic doses of the effective agent orally. Schilling tests were carried out at the end of the therapeutic trial and later iron was administered either orally or as a single intravenous infusion in most patients. No antibiotics were administered.

At the end of 5 to 24 months of therapy with either folic acid or Vitamin B12 orally, 16 patients have been reinvestigated with haematologic and absorption studies and jejunal biopsies.

RESULTS

Clinical Features

Race: 24 of the 27 patients were Indians and 3 were Chinese. The ratio of Indians to Chinese was 9:1.

Sex and Age. 16 were males and 11 were females. The youngest was 16 years old and the oldest 75 years. There was an even distribution throughout this range. Their sex and age distribution corresponded to that of the general hospital population.

Mode of onset and duration of symptoms. The mode of onset was gradual in all but one of the patients. This patient presented with an acute onset of nausea, vomiting and diarrhoea. The duration of symptoms before admission to hospital was less than 6 months in 60% of the patients and between 6 months and 12 months in the rest.

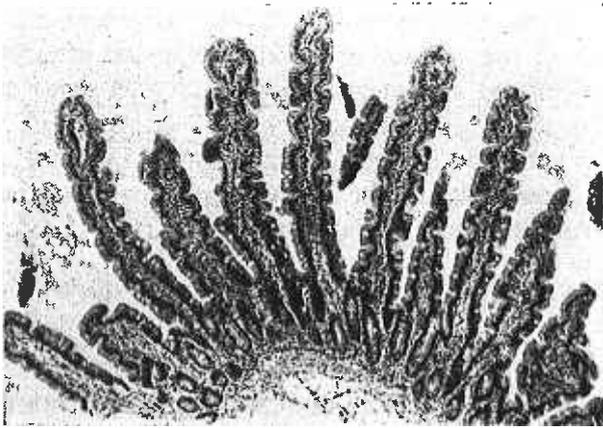


Fig. 1. ($\times 75$). Jejunal biopsy Grade 0. Long slender villi of normal height. (> 350 u). Heavy round cell infiltration of the lamina propria.

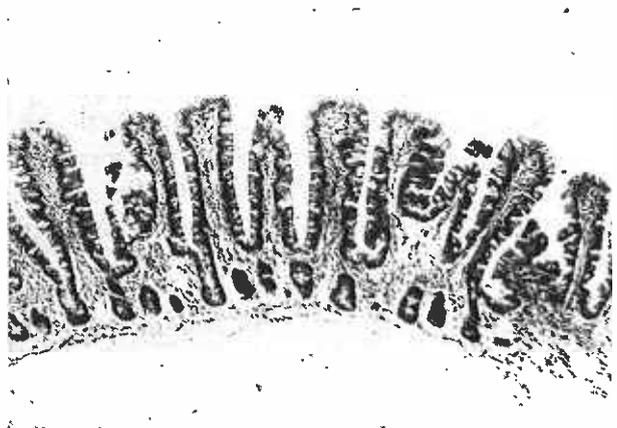


Fig. 2. ($\times 75$). Jejunal biopsy Grade 1. Villi between 300 u and 350 u in height.



Fig. 3. ($\times 150$). Jejunal biopsy Grade 2. Villi between 150 u and 300 u in height.

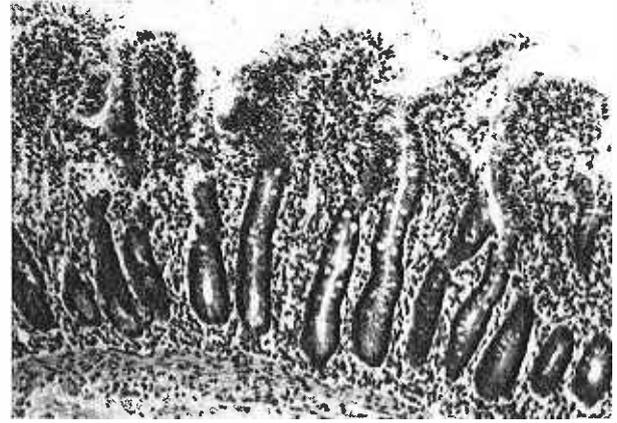


Fig. 4. ($\times 150$). Jejunal biopsy Grade 3a. Short irregular elevations below 150 u in height. Heavy round cell infiltration of the lamina propria and epithelium.



Fig. 5. ($\times 150$). Jejunal biopsy Grade 3b. A flat surface without any villi. Heavy round cell infiltration of the lamina propria and epithelium.

Symptoms: The commonest symptoms in order of frequency were anorexia (in 96%), a sore tongue (in 92%), weight loss (in 85%), diarrhoea (in 51%) and dyspepsia, vomiting after meals, pallor, melanotic pigmentation of the skin of the extremities and slight swelling of the feet and ankles in 30% of the patients. It is noteworthy that half the patients did not have any diarrhoea during their entire illness and some indeed complained of constipation. The initial symptoms which occurred at the onset of the illness varied. The commonest symptoms at the onset were a sore tongue which occurred in 37% of the patients and anorexia which occurred in 26%. Diarrhoea and vomiting occurred at the onset in only 7%. In the remaining patients, the illness began with lassitude, swelling of the legs or pallor and giddiness.

Signs: All the patients had pallor of the mucous membranes and 80% were thin, malnourished and had a smooth atrophic tongue often with painful shallow ulcers along the edges and tip of the tongue. Melanotic pigmentation of the skin occurred in 50% of the patients. Areas which were commonly pigmented were the circumoral area of the face, the palms and soles, and the dorsal aspects of the hands and feet. The degree of pigmentation varied from few scattered brown macules to a uniformly heavy pigmentation. The liver and spleen were slightly enlarged in less than half the patients. No neurological deficit was present except for loss of ankle reflexes in those patients who were above 60 years of age.

HAEMATOLOGICAL FEATURES

The Control Subjects

The haemoglobin levels were within the normal range. The serum iron was normal except for 2 subjects in whom it was subnormal. The serum calcium was subnormal in 2 subjects but normal in the rest. The serum albumin was above 3.5 grams % in all the subjects.

The Patients

All the patients were anaemic and the mean haemoglobin was 5 grams % with a range from 2.2 grams to 10 grams. The marrow was megaloblastic in all the patients. The serum iron before the therapeutic trial was normal in 40%, low in 20% and raised in 40%. After the trial it was low in 95% of the patients, thus indicating a masked iron deficiency in the majority. In the 10 patients in whom serum B12 and folic acid assays were done, low folate levels were found

in all and low B12 levels in 5 patients. Serum calcium estimations were done in only 10 patients. In one patient it was within normal but in the remaining 9 patients it was slightly subnormal and varied between 8 to 9 mg. %. The serum albumin was below 3 grams % in 3 patients, and between 3 grams and 3.5 grams % in 7 patients. In the remaining 17 patients the serum albumin levels were normal.

ABSORPTION STUDIES

Control Subjects

In the 10 Indians studied, no malabsorption occurred in 7. In the remaining 3 subjects, one could not absorb xylose, another could not absorb Vitamin A and xylose and the third could not absorb dietary fat, Vitamin A, and Vitamin B12.

In the 10 Malay subjects, no malabsorption occurred in 6. In the remaining 4 subjects, 1 or 2 of the test substances were inadequately absorbed in the presence of normal absorption of the remaining test substances.

In the 10 Chinese subjects, no malabsorption of the test substances occurred.

Thus partial malabsorption of test substances was detected in 3 out of 10 Indians and 4 out of 10 Malays but in none of the 10 Chinese subjects.

The Patients

D-xylose absorption tests were done in all the patients and malabsorption occurred in 78%. Vitamin A absorption tests were done in 23 patients and malabsorption occurred in 65%. Schilling tests were done in 26 patients and were impaired in 54% and normal in 46%. Steatorrhoea occurred in only 26% (7 patients).

The xylose absorption test has been used as a sensitive screening test in the diagnosis of tropical sprue and in the present series it was the absorption test which was most often impaired. However, the importance of using a number of other tests is demonstrated in the 5 patients with a normal ability to absorb xylose. In these 5 patients, 3 could not absorb Vitamin A, 1 could not absorb Vitamin B12 and 1 had steatorrhoea.

In addition, ova (ankylostoma) were present in the stools of only 3 patients. Cultures of the stools did not reveal any pathogenic organisms. Barium meals did not reveal any strictures or blind loops in any of the patients. Augmented histamine test meals indicated the presence of free acid in 70% of the patients.

SMALL INTESTINAL BIOPSY FINDINGS (Tables I, II and III)

Control Subjects

The mucosal pattern was similar in the 3 ethnic groups. Leaf shaped villi were present in half the patients and ridges were present in the rest. No convolutions or a flat mucosa were present in any of the control subjects.

Results of histological examination were as follows:

Indians: 5 were Grade 0 and 5 were Grade 1. However, even in those who were Grade 0, infiltration of the substantia propria was moderately increased in all but one of the subjects. The mean total mucosal thickness was 557 u and the mean villous height was 397 u.

Malays: 5 were Grade 0 and 5 were Grade 1. Of the 5 with a Grade 0 mucosa, 4 had a moderately heavy round cell infiltration of the substantia propria. The mean total mucosal thickness was 537 u and the mean villous height was 399 u.

Chinese: 4 were Grade 0, 5 were Grade 1, and one was Grade 2. All those with a Grade 0 mucosa had a normal degree of round cell infiltration of the substantia propria. The mean total mucosal thickness was 500 u and the mean villous height was 362 u.

In all 3 ethnic groups, the epithelial cells were columnar with a mean height of 25 u and were normal in appearance. Round cell infiltration was minimal and did not extend above the level of the nuclei of the columnar cells.

The Patients

Mucosal Pattern: Of the 27 patients, 20% had a mucosa composed of leaves, 36% had ridges, 22% had convolutions and 22% were flat. Since the normal control biopsies consisted of leaves and ridges, it is clear that examination with the dissecting microscope revealed abnormalities in 44% of the biopsies.

Histological examination revealed that 8% were Grade 1, 52% were Grade 2, and 40% were Grade 3. Since almost all the normal control subjects had Grade 0 or Grade 1 mucosa, it was possible to conclude that 92% of the jejunal biopsies in the patients were abnormal. Thus in this series, histological examination was more useful than the dissecting microscope in detecting abnormalities of the jejunal mucosa. Ideally of course both techniques should be used in the examination of the jejunal mucosa.

Response to Therapy

A therapeutic trial was carried out with folic acid in 23 patients and Vitamin B12 in 4 patients. All the patients responded with a reticulocytosis and marrow conversion. The haemoglobin usually became normal within 6 weeks. All the patients ultimately received Vitamin B12 when Schilling's tests were carried out and also iron orally or intravenously.

Symptomatic response during the therapeutic trial was striking. 4-5 days after the onset of the trial, anorexia would be replaced by an insatiable appetite, and within a week glossitis, diarrhoea, nausea and vomiting would have disappeared. There was also a steady gain in weight of approximately 1 to 1½ lbs. a week, and most patients increased their weight ultimately by about 20 lbs. The melanotic pigmentation of the skin gradually cleared in about 2 months.

16 patients were reinvestigated after a period of therapy varying from 5 to 24 months.

All the patients were symptomatically well and considered themselves to be in normal health.

Haemoglobin levels were normal in all the patients. The serum albumin was within normal limits in all but the serum iron was subnormal in 4 patients. Intestinal absorption studies revealed a general improvement. Xylose absorption, impaired in 11 patients initially, returned to normal in all after therapy. Vitamin A absorption, impaired initially in 8 patients, returned to normal in 7 patients after therapy. Schilling's tests impaired in 9 patients before therapy, returned to normal in 6 after therapy. Only 1 patient in this group had steatorrhoea and after therapy steatorrhoea was still present although in a lesser degree.

Jejunal Biopsies

One patient had a Grade 1 mucosa and malabsorption of xylose before therapy. After therapy the biopsy was Grade 0 and xylose absorption was normal. 8 patients had a Grade 2 mucosa initially. After therapy the biopsy was Grade 0 in 4 patients and Grade 1 in 2 patients. Malabsorption present before therapy in these patients cleared up after therapy. In the remaining 2 patients no improvement occurred in the jejunal biopsies and only partial improvement in the absorption studies. 7 patients had a Grade 3 mucosa initially. After therapy, in 4 patients the mucosa became Grade 0, and all the malabsorption present initially cleared up. In the

TABLE I

JEJUNAL BIOPSIES (Mucosal Pattern)		
Mucosal Pattern	Normal Controls (%)	Sprue Patients (%)
Fingers	50	Nil
Leaves	50	20
Ridges	Nil	36
Convulsions	Nil	22
Flat	Nil	22

TABLE II(a)

JEJUNAL BIOPSIES (Histology)			
Normal Controls			
Grade	Indians (%)	Chinese (%)	Malays (%)
0	50	40	50
1	50	50	50
2	Nil	10	Nil
3	Nil	Nil	Nil

TABLE II(b)

JEJUNAL BIOPSIES (Histology)	
Tropical Sprue	
Grade	27 Patients (%)
0	Nil
1	8
2	52
3	40

TABLE III

MEAN MEASUREMENTS OF THE MUCOSAE OF CONTROLS AND PATIENTS WITH TROPICAL SPRUE

	No. of Patients	Total Mucosal Thickness (u)	Villous Height (u)	Subvillous Thickness (u)	Villous Width (u)		Columnar Cell Height (u)
					External	Internal	
Controls							
Indians	10	557 (453-632)	397 (342-473)	160 (90-210)	107 (63-126)	58 (27-72)	25 (18-31)
Chinese	10	500 (358-655)	362 (225-473)	138 (90-177)	102 (72-117)	56 (27-72)	24 (20-29)
Malays	10	537 (423-677)	399 (315-508)	138 (80-168)	119 (97-165)	68 (52-87)	24 (18-29)
Tropical Sprue							
Grade 1	2	527 (504-550)	310 (297-324)	217 (180-253)	135 (117-153)	85 (80-90)	25 (24-26)
Grade 2	15	424 (340-495)	244 (180-270)	180 (88-252)	116 (90-171)	73 (45-126)	20 (14-26)
Grade 3	10	356 (279-423)	—	—	—	—	14 (12-18)

3 remaining patients there was partial improvement in the jejunal mucosa and malabsorption tests.

DISCUSSION

There is a growing belief that subclinical tropical sprue is widespread in tropical countries (Klipstein, 1968). Although the number of normal controls was small, our findings that all the Chinese and two-thirds of the Indians and Malays investigated had no malabsorption indicate that this concept of subclinical sprue does not apply to the local population of Singapore. Similarly Troncale *et al* (1967) did not find sufficient malabsorption in 40 Thai control subjects to justify the concept of subclinical sprue in Thailand.

Gardner (1958) in a review on tropical sprue commented that sprue has always been rare in Singapore. Although sprue has been reported in Europeans in Singapore (O'Brien and England, 1964) it has been generally believed that sprue is rare in Asians in this area. However, Seah *et al* (1966) in a report from Singapore described 17 Indian patients who presented with a megaloblastic anaemia. 2 patients had tropical sprue with malabsorption of fat, xylose, and Vitamin B12 and abnormal jejunal biopsies. In the remaining 15 patients, the etiology of the anaemia was considered to be nutritional although intestinal malabsorption was also considered as an underlying cause, since malabsorption of xylose and Vitamin B12 and abnormalities of the jejunal mucosa were found in some of the patients. Similarly Tasker (1961) studying tropical megaloblastic anaemia in Kuala Lumpur, Malaysia, found that the majority of his patients were Indians and that half of them could not absorb Vitamin B12 administered with intrinsic factor. In the present study besides a group of patients with pernicious anaemia, all the patients who were admitted with a megaloblastic anaemia during a 2 year period were found to have malabsorption and abnormal jejunal biopsies and were considered to be suffering from tropical sprue.

24 of the 27 patients were Indians and 3 were Chinese. Of the total population of Singapore, Chinese form 75%, Malays 15% and Indians 6%. It is therefore obvious that Indians are far more susceptible than Malays or Chinese to tropical sprue. Two reasons for this preponderance in Indians may be suggested: (a) Indians unlike the Chinese generally overcook their vegetables and since folates are fairly easily

destroyed by heat, their method of cooking might predispose them to folate deficiency. (b) A genetically transmitted metabolic defect may result in Indians being more susceptible to tropical sprue. This explanation was originally suggested by Althausen *et al* (1962) to account for the rarity of tropical sprue in Negroes in the Caribbean Islands where sprue is common in those of Spanish descent. None of our patients however had a family history of sprue.

Another feature of this group of patients is that only half complained of diarrhoea. This feature is probably due to the fact that the illness began in most of them with anorexia and glossitis. These symptoms forced many to decrease their normal food intake and often to substitute a low roughage diet such as rice porridge for their normal food. The low incidence of steatorrhoea in these patients may be due in part to the absence of easily ingested fat in the form of butter in the high fat diet of the first 8 patients whilst investigations were being carried out. However even if these 8 patients were excluded, the frequency of steatorrhoea would still be only 40% in the remaining patients. Other observers have similarly found an absence of steatorrhoea in 20% to 50% of their patients (Keele, 1946; Jeejeebhoy, 1966).

The presence of jejunal biopsies from normal controls made it fairly easy to evaluate the biopsies from the patients with sprue. However 8% of the patients had a Grade 1 mucosa which can occur in normal controls and only the presence of malabsorption and a change in the jejunal mucosa to a more normal appearance after therapy indicated that these patients were suffering from sprue, and not a nutritional megaloblastic anaemia.

Treatment in these patients has been confined to Folic Acid and Vitamin B12 and on this therapy 11 of the 16 patients reinvestigated have shown a return to normal. However, 5 patients have shown incomplete improvement, and in these patients, antibiotic therapy will probably produce further improvement. Guerra (1965) has shown in 8 Puerto Ricans who had residual gastrointestinal abnormalities despite previous therapy with folic acid that antibiotics on a long-term basis can produce further improvement in the majority.

SUMMARY

Investigations in 30 healthy control subjects of Indian, Chinese and Malay ethnic origin, revealed that all the Chinese and about two-

thirds of the Indians and Malays had a normal capacity to absorb D-xylose, Vitamin A, Co⁵⁸ labelled Vitamin B12 and dietary fat. About a third of the Indians and Malays were unable to absorb one or two of the four test substances used in the absorption studies. Jejunal biopsies did not differ in the 3 ethnic groups and were normal or mildly abnormal.

27 patients presenting with anorexia, a sore tongue and pallor were also investigated. Indians formed the majority of the patients. Diarrhoea occurred in only 51% of the patients. All had a megaloblastic anaemia. Absorption studies revealed malabsorption of xylose, Vitamin A and Vitamin B12 in the majority but steatorrhoea occurred in only 26% of the patients. Jejunal biopsies were mildly abnormal in 8% and moderately or severely abnormal in 92% of the patients. All responded to folic acid or Vitamin B12 therapy. 16 patients were restudied after 5 to 24 months therapy and the majority were found to have improved. Results of investigations and response to therapy indicate that these patients were suffering from tropical sprue. These studies indicate that tropical sprue in Singapore affects Indians mainly and can often present without diarrhoea and steatorrhoea.

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