TROPICAL SPRUE

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In India, a clinical entity of chronic diarrhoea, associated with defective absorption of food, has been recognized since the days of Charaka, a number of centuries B.C. (Baker & Mathan, 1968). During the 19th and early 20th centuries, descriptions of the condition were published by workers in India, the Far East and the Caribbean, and the term “sprue” came into general use (see review by O'Brien, 1971). During the last twenty years important new methods for investigation of the gastrointestinal tract and the haemopoietic system have been developed, and these, together with fresh opportunities for epidemiological studies, have added greatly to our knowledge of sprue and related conditions. Only certain facets of this increased knowledge can be covered here. Other aspects are reviewed by Klipstein (1970) and Baker & Mathan (1970a).

1. The Spectrum of Intestinal Malabsorption

Tropical sprue may be defined as a syndrome of intestinal disease of unknown aetiology, occurring in residents of, or visitors to, certain areas of the tropics, and characterized by malabsorption of two or more test substances. Classically, patients with tropical sprue present with a history of chronic diarrhoea and evidence of multiple deficiency states such as glossitis, angular stomatitis, anaemia and hypoproteinaemia. Investigation shows steatorrhea and other absorptive defects, radiological changes, and changes in intestinal biopsy specimens. However, some patients may present with only one of the complications of malabsorption, such as anaemia, without having had overt gastrointestinal symptoms. In many areas of the tropics, investigation of apparently healthy subjects reveals a high prevalence of minor abnormalities of intestinal structure and function—particularly xylose malabsorption. The literature on this subclinical tropical malabsorption has been summarized by Klipstein (1970). Since the aetiologies of both sprue and subclinical malabsorption are unknown, they can both be defined only in clinical, pathological and biochemical terms. There is clearly a whole spectrum of tropical intestinal disease, ranging from the past with fully developed sprue to the person with only mild intestinal biopsy changes and no detectable malabsorption or isolated mild xylose malabsorption. Patients at either end of the spectrum may be clearly categorized, but in between it may be very difficult to determine whether a given individual is suffering from a mild form of tropical sprue or a severe form of “subclinical malabsorption”. There are some who believe that this spectrum represents differing manifestations of the same disease; however, epidemiological evidence suggests that they are probably separate entities (Baker & Mathan, 1972).

2. Epidemiology

To establish the geographical distribution of tropical sprue, accuracy of diagnosis is essential. Particular care must be taken to exclude intestinal tuberculosis, chronic pancreatitis, gluten-induced enteropathy and parasitic infestations such as Giardia lamblia, Strongyloides stercoralis and Capillaria philippinensis. This calls for thorough gastroenterological investigation and in some cases the true diagnosis may be discovered only at laparotomy or post-mortem examination. There is more than one case of “tropical sprue” in the literature which has ultimately turned out to be a case of secondary malabsorption. On the other hand, many cases of tropical sprue are mis-diagnosed. In 1958, I was told that it did not occur in southern India, but subsequent investigation revealed that sprue was being mis-diagnosed as “abdominal tuberculosis”, “tropical anaemia” or “malnutrition”. Since that time, it has been shown that the prevalence of tropical sprue has been carefully documented from Cuba, Puerto Rico, Haiti, the Dominican Republic, Guatemala, Colombia, India, Singapore, Malaysia, Borneo, Hong Kong and the Philippines. In Africa, south of the Sahara, a number of people have looked systematically for it and have not found it (Foy & Kondi, 1971), but Falaiye (1970) described some patients in Nigeria who had an idiopathic malabsorption syndrome which appeared to be similar to sprue. If these patients were in fact suffering from tropical sprue, then, from the epidemiological view-point, it would be very important to define the areas in Africa in which it occurs.

Sprue occurs in both endemic and epidemic forms and affects all age-groups. It is often considered to be rare in children, but this is probably because paediatricians in the tropics have not been generally aware of the condition. Ashford (1932), in Puerto Rico, found that 10% of his cases were in children under 10, and the occurrence of the disease in children in Puerto Rico has since been confirmed by Santiago-Berrero, Maldonado & Horta (1970). In India, both endemic and epidemic sprue have been documented in children (Mathan, Joseph & Baker, 1969). Figure 1 shows the age-specific attack-rate for over 9000 individuals in a total population of 204160; this was a sample of the population affected in a large epidemic in 1960–62 in Tamil Nadu (previously Madras State) (Baker & Mathan, 1970b). These data were gathered in a retrospective survey, but rather similar age-specific attack-rates have been found in other epidemics studied prospectively, and in each case the attack-rate in children and adolescents has been lower than in adults. In one village, a second outbreak, five years after the first one, showed a maximum attack-rate in children under five

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years of age, suggesting that the rest of the population were relatively immune (Mathan & Baker, 1971).

In epidemics, the spread of the illness within a village presents a fairly characteristic pattern, with clustering of cases in houses or groups of houses. Within a family, several members may fall ill together, or there may be an interval of days or weeks between cases.

Preliminary studies indicate that the prevalence of endemic sprue in southern India is of the order of 14/1000 with an age-sex distribution similar to that of epidemic sprue.

3. Natural History

Epidemics have provided excellent opportunities for studying the natural history of the disease. The outstanding symptom is diarrhoea. In epidemics, about a quarter of the people falling ill develop fever and malarial, either at the same time as the diarrhoea, or preceding its onset by two or three days. During the first week of illness the stools may be watery and contain mucus and, in about one-quarter of cases, flecks of blood. Loud borborygmi, anorexia and a feeling of abdominal distension are also prominent features. These features of the first week of illness are so characteristic that a provisional diagnosis as to the nature of the epidemic can be made early in its clinical course. Some cases have diarrhoea for only a few days and then appear to recover. Others may go on for weeks, months or years, with either continuous or intermittent symptoms. Even in the first week of symptomatic illness, malabsorption and changes in the intestinal mucosal biopsy specimen can be demonstrated. Malabsorption of short duration has been described after several kinds of acute diarrhoeal illness (King & Joske, 1966; Lindenbaum, 1965). However, the distinctive feature of epidemic tropical sprue is that some patients continue for months or years after the initial attack with persisting malabsorption and intestinal biopsy changes. Those with symptoms of short duration have the same epidemiological features as those with symptoms lasting a longer time. It is therefore highly probable that they are all suffering from the same illness (Mathan & Baker, 1971).

As the disease progresses, secondary deficiency states appear. The rate and extent to which these develop depend on the nutritional status of the individual before the onset of the illness, the severity of the malabsorption, and the dietary intake during the course of the disease. The increasing prevalence of deficiencies with increasing duration of symptomatic illness clearly indicates that the deficiencies are the result and not the cause of the disease (Baker & Mathan, 1971; O'Brien & England, 1971).

Descriptions of sprue from different areas of the tropics have differed from each other in such respects as the prevalence of vitamin B12 malabsorption, or folic acid deficiency and of megaloblastic anaemia. A recent study, Tropical sprue and megaloblastic anaemia (1971) (see review, p. 102 of this Bulletin), has compared and contrasted sprue as it occurs in several parts of the world. It was concluded that the cases studied were probably all part of the same syndrome, the natural history in different areas being modified by factors such as the length of residence in the endemic area, the nutritional status, the type of diet and possibly variations in the intestinal bacterial flora.

Spontaneous remission and apparent cure of the disease, even in the absence of "specific" treatment, is well known (Woodruff, 1949). However, the longer the disease persists the less is the likelihood of spontaneous remission occurring (Mathan & Baker, 1970). In patients not receiving medical care the mortality may reach 30%. In the early stages of the disease, and in acute exacerbations, death is most often due to fluid and electrolyte disorders. In cases of longer standing, death may result from anaemia, hypoproteinanaemia and secondary infection.

One patient, who developed diarrhoea at the age of 16, was investigated in this Unit at the age of 15 and found to have typical tropical sprue. Following treatment he had a good remission, his malabsorption disappeared and his biopsy specimen became normal. He remained well for four years, then returned with a recurrence of diarrhoea and was found to have an intestinal lymphoma. Three other subjects have been seen who presented with a history suggestive of sprue for periods ranging from five to nine years, and who, on careful investigation, have been shown to have intestinal lymphoma. This suggests that, as with coeliac disease, chronic tropical sprue may in some way predispose to the development of intestinal lymphoma.

4. Pathology and Pathophysiology

a. Mucoid Anatomy

The histological changes in sprue have been documented in a number of studies (Schenk, Sanloff & Klipstein, 1965; Swanson & Thomassen, 1965). Villus height is usually reduced and crypt depth increased, and in severe cases the glandular layer may come to occupy the whole depth of the mucosa. In mild cases the mucosal cells may show no detectable changes by light microscopy, but in more severe cases the cells, especially those towards the luminal surface, may be flattened and have distorted nuclei and brush borders. There is frequently an increase in the inter-epithelial lymphocytes and at times these may be present in large numbers. In severe cases there is a thickening in the region of the basement membrane, especially marked at the tips of the villi. The lamina propria usually shows an increase in lymphocytes, plasma cells and histiocytes.

Studies by electron microscopy show ultrastructural changes in the mucosal cells, such as distortion of the microvilli, fragmentation of the terminal web, increase in lysosomes, dilatation of the rough endoplasmic reticulum and microchondrial changes. The inter-epithelial lymphocytes at times appear to be clustering around degenerating cells, and
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this may be seen even along the sides of the villi. The base-
ment membrane is normal, to be sure, but especially at the
villus tip, there is a thick amorphous deposit of unknown
composition, and scattered within this there are collagen
fibers and fat droplets (Bruuner, Eidelman & Klopstein, 1970;

b. Intestinal Cell Enzymes

Enzymes of the intestinal mucosal cells in subjects with
sprue have been studied both by histochemical and bio-
chemical techniques. The former have demonstrated slight
decreases in acid phosphatase, lactate dehydrogenase, reduced
NAD and NADP dehydrogenases ("diaphorases"), and more
marked decreases in ATase, succinate dehydrogenase
and cytochrome oxidase (Schenk et al. 1965). In-vitro
estimations, by a number of workers, have shown reduced
activity of a variety of enzymes (Baker & Mathan, 1971)
specifically in the brush border. There is some correlation between the severity of the histo-
logical change and the alteration in enzyme activity (Swamin-
athan, Mathan, Baker & Radhakrishnan, 1970). It must be
emphasized that there is a fundamental problem in measure-
ments of enzymes in intestinal mucosal biopsy specimens.

Enzyme activity is usually expressed either as units per gram
of tissue or units per gram of protein. Since the number of
mucosal cells per unit area depends mainly on the villus
architecture, and since there is no correlation between the
villus shape and the weight or protein content of the biopsy
fragments, it is not possible to determine whether the decreased
values found in patients with abnormal biopsy specimens
reflect the altered villus architecture or whether each individual
cell is defective in terms of its enzyme content. Since the cells
themselves can be shown by light and electron microscopy to
be morphologically abnormal, it is reasonable to expect that
the enzyme levels in individual cells will be altered, but this
has not yet been clearly demonstrated.

c. Intestinal Absorption

Malabsorption of fats, carbohydrates and some vitamins is a
regular feature of tropical sprue. There is probably also
malabsorption of proteins, but this has not been extensively
studied. The pattern of malabsorption appears to differ in
different groups of cases and may even differ in one individual
over the course of time (Baker & Mathan, 1971). Since
knowledge of the normal mechanisms of intestinal absorption
is very fragmentary, it is not surprising that the precise
biochemical lesions responsible for the malabsorption of most
substances remain unknown. The three absorptive defects
that have received most attention are those of fat, vitamin B₁₂
and folic acid.

i. Fat

The normal digestion and absorption of fat depends on
the presence and proper functioning of pancreatic lipase, bile
salts, the mucosal absorptive cells and the transfer of absorbed
lipid to the lymphatics or blood stream. Pancreatic exocrine
function in tropical sprue has usually been assumed to be
adequate but has not been studied in detail. Measurements of
the output of pancreatic enzymes, following a standard
test meal, in 13 southern Indian patients with tropical sprue
have confirmed the normality of pancreatic function
(Balagopal, 1971).

In 20 patients with tropical sprue, chromatography of bile
salts from different levels of the intestine failed to show any
evidence of significant bile-salt deconjugation or dehydroxy-
lation (Kapadia, Radhakrishnan, Mathan & Baker, 1971).

Similar results were found in six Puerto Rican patients, four
of whom also had a reduced bile-salt pool (Turner, Bevan,
Engert, Klopstein & Mulondo, 1970). In further studies in
this Unit, the intake of bile-pool radioactivity caused by a gastric
cholate conjugate has been found elevated in samples of bile
from the jejunum of patients with sprue, but the ratios become
more normal in the ileum. This finding suggests impaired
bile acid reabsorption of bile salts. Further evidence of ileal
malabsorption has been obtained by measuring the faecal
loss of ¹⁴C after feeding ¹⁴C-labelled cholic acid. However,
the extent to which these abnormalities in bile-salt metabolism
are responsible for the steatorrhea of tropical sprue is not
yet clear.

In-vitro studies, employing intestinal mucosa, indicate that
there is also an abnormality of fatty uptake by the cell
and of its intracellular conversion to triglyceride (Baker &
Rao, 1962). Histochemical studies show apparent hold-up of
fat in the thickened basement-membrane region, suggesting
an abnormality of fat transport from the absorptive cells to the
lymphatics (Schenk et al. 1965). Clinical studies further
show that there is some correlation between the extent of the
histological changes seen in jejunal biopsy specimens and the
degree of steatorrhea. Therefore the steatorrhea of tropical
sprue is probably due to a variety of factors, including
bile-salt abnormalities, mucosal cell damage and defective
fat transport from cell to lymphatic.

ii. Vitamin B₁₂

Vitamin B₁₂ malabsorption may be caused either by interference with intrinsic factor production due to a gastric
lesion, or by interference with the normal mechanism of absorption from the lower small intestine. Gastritis is
commonly present, as demonstrated by gastric biopsy findings
and decreased acid secretion. Intrinsic factor assay showed
that in 14% of cases the output is within the range found in
patients with pernicious anaemia (Baker & Mathan, 1971).

The mechanism of intestinal absorption of vitamin B₁₂
is still largely unknown. In patients with the stagnant bowel
syndrome (see Booth, Tabachchali & Mollin, 1968), vitamin B₁₂
malabsorption is common and appears to be related to the
presence of luminal bacteria. In 38 patients with tropical
sprue treated with antibiotics, vitamin B₁₂ absorption returned
to normal in 19. This improvement usually occurred within
a few days, suggesting that bacteria may play an important role
in perpetuating the B₁₂ malabsorption (Baker & Mathan,
1971).

However, in a study of 34 patients with sprue in this
Unit there was no relation between the vitamin B₁₂ absorp-
tion and the number or types of bacteria that could be grown
from aspirates from the jejunum or ileum. In in-vitro studies of
the bacteria obtained from the intestinal aspirates in 15
patients, it was not possible to demonstrate any effect of the
organisms on vitamin B₁₂ bound to intrinsic factor. The
precise way in which antibiotics influence vitamin B₁₂
absorption is therefore still unknown.¹

iii. Folic Acid

Folic-acid absorption is much more difficult to study than
that of vitamin B₁₂ because folic acid is present in food mainly
in the form of polyglutamates. Most tests of intestinal

¹ See also Tabachchali & Booth, Br. med. Bull. 1967, 32, 245-250.
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absorption of folic acid have been performed using \(^{14}C\)-labelled pteroylglutamic acid and measuring urinary or faecal excretion or rise in plasma levels after an oral test dose. Widely different results have been obtained, with malabsorption being present in from 20\% to 80\% of cases (Klipstein, 1919). Some of these differences may be due to variations in the method of performance of the test. When small doses of pteroylglutamic acid are given by mouth there is usually a satisfactory haemopoietic response (Sheehy, Rubin, Perez-Santiago, Santini & Haddock, 1961; Baker & Mathan, 1971), indicating adequate absorption. However, the absorption of food folate is more difficult to study. Jeejeebhoy, Desso, Borkar, Deshpande & Pathare (1968) and Hoffbrand, Necheles, Maldenado, Horta & Santini (1969) measured rises in serum folate levels following the administration of polyglutamates and found evidence that was suggestive of depressed absorption in 18 of 20 patients with tropical sprue. Conjugase appears to be present in normal amounts in the intestinal juice of patients with sprue, but has been found to be reduced in biopsy specimens (Hoffbrand et al. 1969). Studies using labelled polyglutamates synthesized by the solid-phase method may be expected to produce further information on the abnormalities of intestinal absorption of folate. Other factors may also contribute to the folate deficiency of sprue, such as increased faecal losses, decreased intake and increased requirements for folate (Tropical sprue and megaloblastic anaemia, 1971).

5. Aetiology

Tropical sprue may be a syndrome of multiple pathogenesis. The intestinal lesions appear to be damaged by the intestinal mucosa as evidenced by histological changes and alterations in intestinal function. Theoretically, this intestinal damage could be due to a toxin, a deficiency, an infectious agent or a combination of these factors.

a. Toxins and Deficiencies

The occurrence of sprue epidemics might be taken to exclude toxins or deficiencies as the cause of sprue, but both can cause epidemics (e.g. epidemic dropsy and pellagra). No evidence of a toxin has been found. Rancid fats were at one time proposed as a possible cause (French, 1955) but evidence is lacking to support this hypothesis. The frequency of deficiency states in sprue has led to the postulate that it may be a deficiency disease. However, as discussed above, these are almost certainly secondary to the disease process and not of primary aetiological importance.

b. Infectious Agents

The failure to explain epidemics of sprue on a toxic or deficiency basis, the nature of the spread through households and villages, the presence of fever in 25\% of cases at the onset, and an altered age-specific attack-rate in a second outbreak in the same village, all suggest that the disease is caused by an infectious agent.

One of the outstanding features of tropical sprue is the very long latent period which may elapse between a patient’s leaving the tropics, where presumably the disease was acquired, and the onset of his symptoms. Manson-Bahr (1957) reported latent periods of 14–37 years and Mollin (1968) of 22 years. Such a long latent period excludes the possibility that, in these individuals, the disease could have been caused by exposure to a toxin or a deficiency state when the subject was resident in the tropics, and is strongly in favour of an infectiological etiology.

i. Bacteria

The apparent response of some cases of sprue to antimicrobial drugs suggests that bacteria may play a role in its pathogenesis. Culture of stools for known bacterial pathogens has consistently failed to show any difference in isolation rates between patients with sprue and comparable control subjects. In this Unit, in the most recently studied epidemic, the bacterial pathogen isolation rate in adult patients (127 specimens) was 5\% and in controls (251 specimens) was 6\%. Similar results have been obtained in three other epidemics studied.

Studies by light microscopy, and more recently by electron microscopy, have failed to show any bacteria in the mucosal cells or the lamina propria of patients with sprue, so that a bacterial invasion of the mucosa, such as occurs in Whipple’s disease, can be excluded. Quantitative studies of intraluminal bacteria in such patients have produced varying results (Baker & Mathan, 1970a; Gorbach, Banwell, Jacobs, Chatterjee, Mirka, Sen & Mazumder, 1970). In this Unit there was no significant quantitative or qualitative difference between the small intestinal bacterial flora of 34 patients with sprue and control subjects. Even when increased numbers of bacteria are present in the small intestine, these are clearly not the cause of the disease. However, in the presence of existing intestinal damage, the intraluminal bacteria may act as a factor aggravating or perpetuating the mucosal lesion and inhibiting the process of healing. There also remains the rather remote possibility that sprue may be due to an infection with a bacterial pathogen that is as yet unrecognized.

ii. Mycoplasma

With the use of gnotobiotic guinea-pigs, mycoplasma were isolated from the jejunal juice of patients with sprue (Lev, Alexander, Levenson, Hepner, Gerson, Corcino, Janowitz & Herbert, 1969). However, the isolation of mycoplasma does not of itself necessarily imply any aetiological role. In a study of 42 southern Indian patients, P. Bhat (unpublished observations, 1971) has been able to isolate mycoplasma from saliva, but not from intestinal aspirates.

iii. Viruses

Manson-Bahr (1957) suggested that sprue was probably due to infection with a virus "... like the herpes virus, which can maintain itself unchanged for many years in the human body ...". The role of viruses in diseases of the gastro-intestinal tract has been studied by several groups of workers, in both animals and man. In infant mice, rotavirus infection causes a fatty diarrhoea, but this is due to involvement of the pancreas rather than to a lesion of the small intestine (Walters, Joske, Leuk & Stanley, 1963). In baby pigs, the "transmissible gastroenteritis virus" produces a lesion which histologically bears some resemblance to that seen in patients with tropical sprue, but affected animals either die or recover after about a week (Marnopot & Whitehair, 1967). In monkeys (Macacus radiata), rotavirus type 3 infection, given either orally, intragastrically or intravenously, produced diarrhoea in 13 of 17 animals. This diarrhoea lasted up to two weeks and, even in animals inoculated intravenously, virus could be recovered from rectal swabs for up to 12 days (Massillamony &
Recently, inoculation of monkeys with a virus as yet unidentified produced diarrhea which lasted a number of weeks and which was associated with intestinal malabsorption (E. A. Boulier, T. J. John and R. P. Masaslimany, unpublished observations, 1971).

Epidemics of gastroenteritis have been caused by a variety of viruses (Reimann, 1963). However, none of these epidemics has been described as being associated with intestinal malabsorption. Sabin (1956) described a reovirus infection in children that produced a self-limiting "steatorrheic enteritis". Scheel, Arnstein & Green (1964) described biopsy changes and steatorrhea in four of five adults with infectious hepatitis, but the xylex excretion in these patients was normal, suggesting that the steatorrhea may have been due to the hepatitis rather than to the jejunal involvement. Therefore, although in both animals and man there is good evidence that viral infections can cause alterations in small intestinal structure and function, these conditions appear to be self-limiting, and there is as yet no parallel to those cases of tropical sprue that persist for months or years after the initial illness.

Attempts to isolate a virus from patients with sprue have so far been unsuccessful (Bayless, Guaridiola-Roiger & Weheby, 1966). In a recent epidemic in a southern Indian village, the viral flora of 116 patients in the first two weeks of illness and of 280 controls was investigated. Stool or rectal swab specimens were inoculated into cultures of primary monkey kidney and the HEP-2 cell line, and into suckling mice. A total of 47 isolates was obtained but the isolation rate was the same in controls and patients. Jejunal juice, intestinal biopsy specimens and stools obtained from eight patients with the first week of illness and inoculated into monkey-kidney cultures and into HEP-2, WI-38 and Vero cell lines, suckling mice and guinea-pigs also failed to produce any isolates (E. A. Boulier, unpublished observations, 1971).

Thus, although the circumstantial evidence in favour of a virus as the cause of tropical sprue is very strong, the usual techniques of viral isolation have so far not been successful, and other techniques are required. Further major advances in our understanding of tropical sprue await a solution to this problem of its aetiology.

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