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Idiopathic Small Intestinal Disease in the Tropics

S. J. BAKER

1. HISTORICAL INTRODUCTION

For over two and one-half thousand years, small intestinal disease has been recognized as a health problem on the Indian subcontinent. Caraka, somewhere between the 13th and 6th centuries B.C., described "grahani vyadhi" or "small intestinal disease" in the following terms:

> The patient even if not emaciated feels weakness and langour. . . . Food instead of contributing to growth issues out of the body in an upward or downward course . . . .whatever food a person afflicted with grahani disease takes is improperly digested. The person repeatedly evacuates stools that are sometimes watery, sometimes dry, sometimes consisting of undigested matter and frothy, the downward wind making a loud noise at the time (Caraka Samhita, 1949).

The first description of tropical small intestinal disease in European medical literature appears to be that of "apthoides chronica" in Barbados (Hillary, 1759). A somewhat similar condition was observed among the Spanish in Puerto Rico between 1771 and 1776 (Abbad y Lasierra, 1959). During the 19th and first half of the 20th century, numerous reports appeared of an illness characterized by chronic nonbloody diarrhea and anemia from the Indian subcontinent, China, Indochina, Java, Cuba, and Puerto Rico. Various names were given to this illness by different investigators, including "hepatic flux" (Ballingall, 1818), "chronic diarrhea of India and the tropics" (Fayrer, 1881), "diarrhea alba" (MacLean, 1886), "psilosis" (Thin, 1897), and "hill diarrhea" (Grant, 1854).

S. J. BAKER • Department of Medicine, Section of Gastroenterology, University of Manitoba, St. Boniface Hospital, Winnipeg, Manitoba R2H 2A6, Canada.

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Manson (1880) appears to have been first to apply the term "sprue" to this syndrome. This is apparently an Anglicized form of the Dutch word "sprouw," which was applied by Ketelaer (1669) to a disease in Belgium characterized by aphthous ulceration and chronic diarrhea. The word "tropical" was added subsequently to distinguish it from the "nontropical" variety, which is now known as celiac disease or gluten-induced enteropathy.

In the first half of the 20th century, the work of Bahr (1915), Fairley (1930), Suarez (1938), and others established that tropical sprue was primarily a gastrointestinal disorder associated with steatorrhea, megaloblastic anemia, and multiple deficiency states. Bahr also demonstrated the presence of histological abnormalities in the small intestinal mucosa (Bahr, 1915), but the existence of these abnormalities continued to be a matter of controversy for many years.

With the advent of modern investigational techniques, there has been a renewed interest in, and increased understanding of, small intestinal diseases occurring in the tropics. Most of the remainder of this review will be confined to this period. For further historical details, the reader is referred to reviews by O'Brien (1971) and Cook (1978a).

2. THE SPECTRUM OF SMALL INTESTINAL DISEASE IN THE TROPICS

Residents of the tropics may be afflicted by almost any of the diseases of the small intestine that occur in the temperate areas of the world, although the relative prevalences of different diseases may vary widely. For example, in a detailed study of over 1500 patients with small bowel disease in South India over a 20-year period, this reviewer saw only four cases of Crohn's disease of the small bowel, whereas working in a similar sized institution in Canada, as many new cases may be seen in a month.

It is not the purpose of this review to deal with these conditions, which are well covered in the standard temperate-zone-oriented text books of medicine. However, in addition to these well-known temperate diseases, people living in, or visiting, the tropics are liable to develop a variety of diseases of the small intestine that are either relatively less common, or perhaps never seen, in residents of temperate climes. These latter include diseases whose etiology is well recognized such as acute bacterial infections, intestinal tuberculosis, and parasitic disorders such as hookworm, giardiasis, strongyloidiasis, and capillariasis. But over and above these clearly identifiable disorders, there is a wide spectrum of ill-defined disease of unknown origin, ranging from mild asymptomatic abnormalities of structure and function of the small bowel to a full-blown picture of intestinal malabsorption presenting with chronic diarrhea, weight loss, mega-

loblastic anemia, steatorrhea, hypoproteinemia, vitamin deficiencies, and multiple absorptive defects.

Unfortunately, there is a great deal of confusion in the literature regarding the nomenclature of conditions within this spectrum of disease. Such confusion is probably inevitable for as long as ignorance of etiology and pathogenesis persists. For the purpose of this review, the spectrum of tropical idiopathic small intestinal disease will be arbitrarily divided into a consideration of the mild asymptomatic abnormalities of structure and function, hereafter termed "tropical enteropathy" (Baker and Mathan, 1972), and symptomatic disease associated with malabsorption of two or more substances, hereafter referred to as "tropical sprue" (Klipstein and Baker, 1970). The borderland between these two is even more obscure and can probably only be profitably explored when we understand the two entities more fully.

3. TROPICAL ENTEROPATHY

3.1. Occurrence

With the application of jejunal biopsy studies, it soon became evident that morphological abnormalities of the jejunal mucosa were widely prevalent in asymptomatic people living in a number of tropical or developing countries including India (Baker *et al.*, 1962; Jeejeebhoy *et al.*, 1966), Pakistan (Russell *et al.*, 1966), Bangladesh (Lindenbaum *et al.*, 1966a), Thailand (Sprinz *et al.*, 1962; Troncale *et al.*, 1967), Vietnam (Sheehy *et al.*, 1965, 1968; Colwell *et al.*, 1968), Singapore (England and O'Brien, 1966), Australia (in Aboringinals) (Walker-Smith and Reye, 1971), Iran (Nasr *et al.*, 1976), Liberia (Rhodes *et al.*, 1971), Egypt (Halsted *et al.*, 1969), Uganda (Banwell *et al.*, 1964; Cook *et al.*, 1969), Rhodesia (Zimbabwe) (Thomas *et al.*, 1976), Zambia (Cook *et al.*, 1973), Nigeria (Falaiye, 1969, 1970), Haiti (Klipstein *et al.*, 1966a; Brunser *et al.*, 1970), the Dominican Republic (Klipstein *et al.*, 1973a), Puerto Rico (Angel *et al.*, 1963; Robins *et al.*, 1967; Swanson *et al.*, 1966; Klipstein *et al.*, 1972), Mexico (Garcia, 1968), Venezuela (Roche and Layrisse, 1966), Guatemala (Schneider and Viteri, 1972), and Peru (Perea *et al.*, 1978).

3.2. Pathology

With increasing degrees of damage, the intestinal villi assume a "leaf," "ridge," or "convoluted" pattern, and finally, in the most damaged mucosa (e.g., in celiac disease), the surface is "flat" and devoid of villi (Booth *et al.*, 1962). In the tropics, fingerlike villi are rare, and the predominant form, in

apparently healthy individuals, is "leaf" or "ridge" shaped (Baker *et al.*, 1962), reflecting widespread mild damage of the intestinal mucosa. On histological section, ridges cut along their long axes give the appearance of "broad flat" villi. In addition, there is an increase in the depth of the crypts with a corresponding reduction in the height of the villus and an increase in the cellular infiltrate in the lamina propria and in the number of lymphocytes between the epithelial cells (Baker *et al.*, 1962; Swanson and Thomassen, 1965; Cook *et al.*, 1969; Halstead *et al.*, 1969; Haghighi and Nasr, 1975). Electron microscopic studies confirm the above light microscopy findings and, in addition, show an increase in lysosomes in the supranuclear region of the enterocytes (Brunser *et al.*, 1970; Mathan *et al.*, 1975a). Mathan *et al.* (1975a) also found occasional dark-staining degenerating epithelial cells in the upper two-thirds of the villus, away from the zone of extrusion.

In order to try to overcome some of the difficulties inherent in the interpretation of jejunal biopsies by different investigators, photographs of the dissecting and light microscopic appearance of seven jejunal biopsies from apparently healthy asymptomatic south Indian subjects with varying degrees of enteropathy were circulated to 42 different investigators in various countries. Respondants were asked to indicate the approximate frequency with which such a biopsy might be seen in apparently healthy people in their area. The results confirmed that both dissecting and light microscopic abnormalities were much more prevalent in apparently healthy subjects living in the tropics than in similar people living in temperate climates (Baker, 1973).

3.3. Intestinal Function

In addition to architectural and histological changes in the intestinal mucosa, there is often some evidence of impaired intestinal function. The test most frequently employed to study small bowel function has been the xylose tolerance test. Decreased xylose absorption has been demonstrated by a number of investigators (Sprinz *et al.*, 1962; Angel *et al.*, 1963; Lindenbaum *et al.*, 1966a,b; Russell *et al.*, 1966; Banwell *et al.*, 1967; Robins *et al.*, 1967; Troncale *et al.*, 1967; Falaiye, 1969; Halsted *et al.*, 1969; Baker and Mathan, 1972; Einstein *et al.*, 1972; Schneider and Viteri, 1972; Klipstein *et al.*, 1972, 1973a, 1976; Thomas *et al.*, 1976).

In some countries a small proportion of apparently normal subjects has been found to have steatorrhea (Robins *et al.*, 1967; Cowan *et al.*, 1971; Cowan, 1972; Troncale *et al.*, 1967; Baker and Mathan, 1972). In south India, even in subjects without steatorrhea, the mean fat excretion is higher than that of healthy control subjects in England, suggesting a widespread mild impairment of fat absorption (Baker and Mathan, 1972).

Malabsorption of vitamin B_{12} has been found in from 3 to 50% of appar-

ently normal subjects living in the tropics (Lindenbaum *et al.*, 1966a,b; Troncale *et al.*, 1967; Klipstein, 1971; Baker and Mathan, 1972; Klipstein *et al.*, 1972, 1973a,b; Nasr *et al.*, 1976). Increased losses of fecal nitrogen have been reported in some individuals (Nasr *et al.*, 1976; Klipstein *et al.*, 1972, 1973a; R. E. Schneider and F. E. Viteri, personal communication, 1976), and reduced absorption of glycine and glycyl-glycine has been demonstrated by intestinal perfusion studies in apparently healthy southern Indian villagers as compared with English subjects living in England (Hellier *et al.*, 1976).

The nutritional consequences of these abnormalities of absorption have not been fully investigated. R. E. Schneider and F. E. Viteri (personal communication, 1976) determined energy balances in patients with tropical enteropathy and were able to demonstrate a significant energy wastage. If this be the case whereever this lesion is prevalent, then it may have important nutritional consequences, especially for those already existing on a marginal energy intake.

3.4. Pathogenesis

3.4.1. Epidemiology

Human fetuses in India (Baker *et al.*, 1962) and Africa (Stanfield *et al.*, 1965; Cook *et al.*, 1969) have fingerlike villi. The architectural change in the villi must therefore be acquired. From study of postmortem specimens, it has been shown that the lesion appears in the first few months of life and is maximal in older children and adults (Chacko *et al.*, 1969).

Impairment of xylose absorption has also been shown to be prevalent in preschool children in south India (Baker and Mathan, 1972) and Bangladesh (Einstein *et al.*, 1972; Harper, 1972), confirming the early onset of abnormalities in intestinal function. In south India, xylose malabsorption was found to be more prevalent in apparently healthy subjects living in villages than in "hospital control subjects" largely of urban origin (Baker and Mathan, 1972). In Guatemala, the prevalence of xylose malabsorption was highest in males living in rural areas, lower in a comparable group of men serving in the army, and lowest of all in urban middle class students of a military academy (Schneider *et al.*, 1974). In a rural village in Guatemala, the prevalence of enteropathy significantly decreased with the introduction of a protected water supply (R. E. Schneider and F. E. Viteri, personal communication, 1976). Further, when inhabitants of tropical countries migrate to temperate climates, the intestinal lesion improves (Gerson *et al.*, 1971a; Lindenbaum *et al.*, 1972; Klipstein and Falaiye, 1969). There is thus evidence that the lesion of tropical enteropathy in the indigenous population is acquired early in life and is more prevalent in rural populations who, presumably, are more exposed to some dietary or environmental "toxin."

Studies of migrants also support the dietary or environmental toxin theory. When expatriates from temperate climates move to tropical countries, they tend fairly rapidly to develop an intestinal lesion similar to that of the local inhabitants (Keusch *et al.*, 1970; Lindenbaum *et al.*, 1966b; Sheehy *et al.*, 1965, 1968; S. J. Baker and V. I. Mathan, unpublished observations, 1970). Moreover, in Bangladesh, Lindenbaum *et al.* (1966a) have shown that the enteropathy was worse in expatriate Peace Corps volunteers who were exposed to the local environment than in expatriate embassy personnel who led a more sheltered existence. These changes in expatriates tend to regress when the individual returns home (Lindenbaum *et al.*, 1971; Sheehy *et al.*, 1965, 1968).

In Puerto Rico there appears to be a seasonal pattern to the prevalence of tropical enteropathy, with a peak occurring in the month of January (Klipstein *et al.*, 1972; Klipstein and Corcino, 1974). However, these studies were only carried out over a short period of time, and it is therefore not certain that this is an annual occurrence.

All of the above studies strongly suggest that dietary or environmental factors of some sort are responsible for tropical enteropathy, but precisely what these factors are is not clear. A number of different conditions have been described that are prevalent in tropical areas and are known or suspected to be capable of damaging the intestine, including nutritional deficiences and infectious agents.

3.4.2. Nutritional Factors

A variety of nutritional deficiencies have been described, often on rather insubstantial evidence, as causing morphological changes in the gastrointestinal tract of man (Baker, 1977). However, the abnormalities of tropical enteropathy are seen even in subjects eating a good diet (Baker *et al.*, 1962; Lindenbaum, 1968), and, as noted above, they may occur in well-nourished expatriates shortly after their arrival in the tropics. It therefore seems improbable that dietary deficiencies *per se* account for the lesion.

Klipstein and Corcino (1974) have suggested that seasonal changes in dietary practices such as an increased intake of long-chain unsaturated fatty acids might alter the intestinal microenvironment and favor colonization by enterotoxigenic bacteria (see Section 3.4.4), thus accounting for the seasonal pattern of prevalence of tropical enteropathy seen in Puerto Rico.

3.4.3. Parasites

Parasites such as *Strongyloides stercoralis* (Stemmermann, 1967), *Giardia lamblia* (Yardley *et al.*, 1964), *Capillaria philippinensis* (Whalen *et al.*, 1969), coccidiosis (Brandborg *et al.*, 1970), and cryptosporidiosis (Meisel *et al.*, 1976)

can produce intestinal damage in man sufficient to cause a frank malabsorption syndrome, and it is possible that milder infestations might produce lesser degrees of damage. However, the distribution and prevalence of these parasites are not such as to explain the ubiquitous nature of tropical enteropathy. It has been claimed that hookworm infestation can cause morphological changes in the small intestine (Sheehy *et al.*, 1962a; Tandon *et al.*, 1966a; Chuttani *et al.*, 1967), but unfortunately these studies were done in areas where tropical enteropathy is prevalent, and more critical studies suggest that hookworm plays little or no role in the production of these lesions (Layrisse *et al.*, 1964; Burman *et al.*, 1970). Moreover, the lesion appears in very young children before they have contracted hookworm or other parasitic infestations (Chacko *et al.*, 1969).

3.4.4. Bacteria

Animals raised in a bacteria-free environment have taller villi, shorter crypts, and less cellular infiltration of the lamina propria in the small intestine than their conventionally raised counterparts (Abrams *et al.*, 1963). Several studies of the bacterial flora of the intestine of residents in the tropics, especially those living in rural areas, have shown increased numbers of bacteria in the upper jejunum of at least some subjects (Gorbach *et al.*, 1970; Bhat *et al.*, 1972, 1980; Mata *et al.*, 1972a; Klipstein *et al.*, 1973a). However, it should be noted that subjects in temperate zones who have bacterial overgrowth associated with a stagnant loop syndrome do not display histological abnormalities in their intestinal mucosa (Donaldson, 1965) or at most have a mild patchy lesion (Ament *et al.*, 1972). Furthermore, bacterial contamination of the bowel tends to increase towards the ileum, yet the lesion of tropical enteropathy is, if anything, more marked proximally than distally (Baker *et al.*, 1962; Chacko *et al.*, 1969). It must, however, be pointed out that bacteria that can be aspirated from the gut lumen do not necessarily reflect the total population of intestinal bacteria, since there may be quite a different flora closely adherent to the mucosa (Bhat *et al.*, 1980). Moreover, failure to isolate a significant number or specific type(s) of organism at one point in time does not rule out the possibility of repeated transient bacterial infections of the upper intestine.

Acute infections with specific bacterial pathogens may be followed by a temporary period of malabsorption (Lindenbaum, 1965). Repeated enteric infections may possibly produce a more long-lasting derangement of intestinal structure and function (Gerson *et al.*, 1971a; Lindenbaum, 1973). In south India (Baker and Mathan, 1972) and in Bangladesh (Einstein *et al.*, 1972), a correlation was observed between the number of episodes of diarrhea and the results of xylose absorption tests. In Rhodesia, (Zimbabwe), Thomas *et al.* (1976) have noted that enteropathy is more marked in individuals of lower socioeconomic status and suggest that this is because of their greater exposure to repeated

gastrointestinal infections. On the basis of observations in Puerto Rico, it has been suggested that repeated infections with organisms not usually classified as enteric pathogens but which elaborate an enterotoxin may be responsible for the intestinal damage (Klipstein and Corcino, 1974; Klipstein and Schenk, 1975a; Klipstein, 1979).

Another possible mechanism whereby bacteria can damage the intestine is by deconjugation of bile salts. Apparently healthy Guatemalan children with tropical enteropathy have been found to have deconjugated bile salts in the upper jejunum (Schneider and Viteri, 1974). However, this has not been found in other parts of the tropics where bile salts have been studied (Cassells *et al.*, 1970; Kapadia *et al.*, 1971a; Desai *et al.*, 1972; Bevan *et al.*, 1974).

Finally, Cook (1972) has suggested that acute or chronic systemic bacterial infections may affect intestinal function. The mechanism by which this occurs is not clear, but if, indeed, the enterocyte itself is affected, then it is possible that repeated systemic infections may also play a role in the production of tropical enteropathy.

3.4.5. Viruses

Inhabitants of the developing regions of the world are exposed to a variety of viral agents from the time of birth. Some, such as rotavirus, produce an acute enteritis from which the patient usually recovers with little or no sequelae. However, many other viral agents may be found in the stools. Thus, viral isolates can be obtained from 20% of stool specimens from Guatemalan village children under 3 months of age (Mata *et al.*, 1972b) and in 58% of randomly collected stools from south Indian preschool children (T. J. John, personal communications, 1972). Other viruses, which may not be cultivatable, may be seen by electron microscopic examination of the stools (Mathan *et al.*, 1975b). The role, if any, of these agents in the pathogenesis of tropical enteropathy is unknown.

3.5. Summary and Conclusions

Enteropathy characterized by asymptomatic abnormalities of small intestinal structure and function is widely prevalent in tropical and developing countries. While the intestine may be affected by many insults, there is considerably circumstantial evidence that microbial contamination of the intestine plays an important role in initiating and perpetuating the lesion. This evidence includes the facts that the enteropathy occurs early in life or soon after arrival in the tropics, it is more prevalent in children who have more bouts of diarrhea, it is more prevalent in rural populations, it is more prevalent in expatriates exposed to the local environment and less in those who are less exposed and, finally, it tends to clear up when individuals move to a more sanitised environment. The precise

organisms involved, bacterial, viral, or others, and the way in which they produce damage remain to be discovered. The effect of this lesion on the nutritional status of tropical inhabitants has not been fully elucidated, but it may well play a significant role in those living on already marginal food intakes. One hopes that as levels of hygiene and sanitation improve in the developing world, this condition will become less prevalent.

4. TROPICAL SPRUE

As noted above, the term tropical sprue is arbitrarily defined as a syndrome occurring among residents of, or visitors to, the tropics, usually characterized by diarrhea and other symptoms together with malabsorption of two or more substances.

4.1. Epidemiology

4.1.1. Geographical Distribution

One of the interesting and as yet unexplained facts about the syndrome of tropical sprue is its geographical distribution. It is widely prevalent on the Indian subcontinent (Bahr, 1915; Baker and Mathan, 1968a). It has also been described in many parts of southeast Asia such as Burma (Walters, 1947; Ayrey, 1948), Thailand (Juttijudata *et al.*, 1969; O'Brien and England, 1966, 1971), Vietnam (Sheehy *et al.*, 1965; Miller *et al.*, 1974; Pittman and Pittman, 1976), Hong Kong (Webb, 1956; O'Brien and England, 1971), Malaya, Singapore, Borneo (O'Brien and England, 1971), Indonesia (Sheehy *et al.*, 1965; O'Brien and England, 1971), and the Philippines (Olson and Layne, 1947; Sparberg *et al.*, 1967). It used to be prevalent in China (Begg, 1912), but this reviewer has been unable to find any recent reference to its occurrence there. It is also prevalent in areas of the Caribbean and neighboring countries such as Puerto Rico (Ashford, 1932; Suarez, 1938; Rodriguez-Molina, 1943; Bayless *et al.*, 1968), the Dominican Republic (Klipstein and Falaiye, 1969), Haiti (Klipstein *et al.*, 1966a), Cuba (Lopez *et al.*, 1949), Guatemala (Klipstein and Falaiye, 1969), Costa Rica (Duran, 1924), Venezuela (Beker *et al.*, 1961), and Colombia (Ghitis *et al.*, 1967).

Although tropical sprue is prevalent in many tropical countries, it is alleged to be rare or absent in others, notably Jamaica and the African subcontinent south of the Sahara. It is, of course, always difficult to exclude the presence of a specific disease in a given geographical area unless a definitive search is made for it by someone familiar with the condition. It is even more difficult to exclude what is probably a syndrome that can be mimicked by a number of other conditions.

Gardner (1958) states that tropical sprue does not occur in Jamaica but does not give any reference to support that statement. P. Milner (quoted in Klipstein, 1971), studying megaloblastic anemia in adults in Kingston, Jamaica, was unable to identify any cases of tropical sprue. Further, although other cases of malabsorption have been investigated at the University Hospital of the West Indies, no case of tropical sprue was recognized in the 20-year period from 1950 to 1970 (E. K. Cruikshrank, quoted in Ashcroft, 1970).

Foy and Kondi (1971) reviewed reports of malabsorption syndrome in Africa and concluded that there was no good evidence in the literature for the occurrence of tropical sprue in sub-Saharan Africa. Banwell *et al.*, (1964, 1967), Foy and Kondi (1971), and Cook (1974a) specifically looked for tropical sprue, particularly among patients with a malabsorption syndrome and/or megaloblastic anemia, but were unable to document any cases. On the other hand, Falaiye (1970) described nine Nigerian subjects, and Moshal *et al.* (1975) 24 South African subjects who had a malabsorption syndrome compatible with a diagnosis of tropical sprue. Thomas and Clain (1976) described 31 cases seen in Salisbury, Rhodesia (Zimbabwe), over a 15-month period, that seem to closely resemble the syndrome as seen in the Caribbean. These latter authors point out that the prevalence and geographical distribution of the syndrome in Africa have been inadequately studied—an opinion with which this reviewer is in complete agreement.

There have been four reports of a syndrome "resembling tropical sprue" in subjects who have always lived in temperate zones (Cooke *et al.*, 1963; Kendall and Bayley, 1971; Goldstein *et al.*, 1972; Montgomery *et al.*, 1973). In some of these subjects, the condition appears to have started with a gastroenteritislike illness, whereas in others the onset was insidious. Although these cases certainly bear some similarity to the tropical sprue syndrome, the rarity with which they have been described from centers where sophisticated investigation of gastrointestinal disease is common is in marked contrast to the high prevalence in many regions of the tropics, and for the time being, at least, it would seem prudent to keep these separate from the syndrome of tropical sprue and to confine that term to disease developing in residents of, or visitors to, the tropics.

4.1.2. Epidemic Sprue

One of the fascinating features of tropical sprue is the tendency for it to occur in epidemics. The first suggestion of an epidemic is Hillary's account of the disease in Barbados (Hillary, 1759). This apart, most of the recorded epidemics have been in south India, northwest India, northeast India, East Pakistan (now Bangladesh), and Burma (Baker and Mathan, 1968a). Stefanini (1948) was able to study an epidemic that occurred over a 4-year period among Italian prisoners of war in a camp in the Yol-Kangara valley in the Himalayan foothills.

In south India there are unpublished accounts of at least five epidemics from 1929 to 1940 in the records of the Madras State Health Services (Baker and Mathan, 1968a), and within the last 20 years we have studied several other epidemics, details of some of which have been published (Baker *et al.*, 1963; Mathan *et al.*, 1966; Baker and Mathan, 1968a, 1970, 1972; Mathan and Baker, 1968, 1970, 1971). The smallest of these was an epidemic in an isolated household in which 16 out of 27 members where affected (Mathan *et al.*, 1966), and the largest involved an estimated 100,000 individuals with a case fatality rate of around 30% (Baker and Mathan, 1970). Contrary to the findings of Stefanini (1948), there was no clear-cut seasonal pattern.

Most of the epidemics we have studied have had a number of features in common, such as a higher age-specific attack rate in adults, an epidemic curve that peaks in 2–3 months and then tails off over a period of 12 months or more, a clustering of cases in space and time within the village, and, in one case, evidence that in a second epidemic wave, there was a higher attack rate in children under 5 who had not been exposed at the first epidemic 5 years earlier (Mathan and Baker, 1971). In all of the epidemics, many subjects have only a short-lived diarrhea which clears within a few days or weeks without any apparent residual damage, whereas others go on with diarrhea for months or years, with either a continuous course or one punctuated by remissions and relapses, and develop all the features of classical tropical sprue. The epidemiologic features are the same, irrespective of the duration of symptoms, suggesting that the same "agent" is responsible for both the short self-limiting condition and the full-blown syndrome of tropical sprue.

Dean and Jones (1972) and Jones *et al.*, (1972) describe seasonal epidemics of gastroenteritis at an American military base in the Philippines. They postulated a water-borne infectious agent as the cause of the epidemic but were unable to isolate any agent. Usually, the illness cleared up in a few days, but in 22% of patients it lasted more than 3 weeks and was accompanied by xylose malabsorption, decreased mucosal disaccharidases, and mild changes in intestinal biopsy and small bowel X-ray findings. Symptomatic relief was obtained by treatment with tetracycline and folic acid. In the present state of knowledge, it is impossible to know if these epidemics bear any relation to the epidemics of sprue seen in India.

4.1.3. Endemic Sprue

In India (and possibly the Philippines), tropical sprue occurs in both endemic and epidemic forms, but in other countries only the endemic form has been described. There are no good studies of the epidemiology of endemic sprue. It is usually thought of as being a disease of adults, but Ashford (1932) in Puerto Rico found that 10% of his cases were in children under 10, and more recently

well-documented descriptions have been published of the condition in children in south India (Mathan *et al.*, 1969), north India (Mehta *et al.*, 1968), and Puerto Rico (Santiago-Borrero *et al.*, 1970).

In a study of 21,000 people in 25 villages in south India, the age-specific attack rate of chronic diarrhea clinically resembling tropical sprue was found to have very similar pattern to the age-specific attack rate in epidemic sprue; i.e., all age groups were affected, but there was a lower incidence in adolescents (0.6%) and a higher incidence among those over 30 (2.2%) (Baker and Mathan, 1972).

In Puerto Rico, Sheehy *et al.* (1965) found an annual incidence of sprue among North American adults (military personnel and their families) of approximately 8%—a rate which must almost qualify for description as an "epidemic." Although some children had intermittent diarrhea, none were included in the study.

Little is known about the epidemiology of the tropical sprue in the indigenous populations in the Caribbean region. In both Puerto Rico and Cuba there is some evidence that the incidence has declined (Roderiguez-Molina, 1943; Milanes, 1960; Sheehy *et al.*, 1965). Klipstein and Corcino (1974), studying 27 patients hospitalized with tropical sprue, found a peak onset of symptoms in the winter months of December to February. These authors postulate that this may be related to changes in dietary patterns.

Further clarification of the true geographical distribution and epidemiology of epidemic and endemic forms of tropical sprue must await an understanding of the etiology, or etiologies, of the syndrome and the development of diagnostic and screening tests suitable for population studies.

4.2. Clinical Features

The vast majority of patients with tropical sprue present with a history of chronic diarrhea. This may develop insidiously so that the individual is unable to determine with any accuracy when the condition began. In other subjects, the first symptom may be a well-remembered attack of acute watery diarrhea, sometimes accompanied by mild fever, nausea, and vomiting. Initially this may be diagnosed as gastroenteritis, but, unlike the usual attack of gastroenteritis, the illness persists, the stools become less fluid, and gradually symptoms and signs of secondary deficiences develop. It is not uncommon for subjects who present in this way to have been associated with others who were similarly affected but in whom the initial illness cleared up without the development of evident malaborption (O'Brien and England, 1966, 1971; Baker and Mathan, 1968a; Mathan and Baker, 1971). With either mode of presentation the diarrhea may be continuous or remittant. Associated with the diarrhea, there are often other gastrointestinal symptoms such as anorexia, abnormally loud borborygmi, abdominal fullness or

distension, nausea, and vomiting. Occasionally, patients present without preceeding gastrointestinal symptoms but with one of the complications of malabsorption such as megaloblastic anemia, and no history of diarrhea can be elicited. Initial symptoms may develop within 5–6 days of entering an epidemic area (Baker and Mathan, 1968a) or may not appear for many years after leaving the tropics (Manson-Bahr, 1957; Mollin and Booth, 1968).

Physical examination in the early stages is usually noncontributory, although loud borborygmi and abdominal distension may be present, and, in thin people, there may be visible small intestinal peristalsis. At any stage of the illness, patients with severe diarrhea may develop dehydration, hyponatraemia, and hypokalaemia. As the condition continues, multiple deficiency states may develop, leading to anemia, dependant edema, ascites, glossitis, angular stomatitis, xerosis conjunctivae, peripheral neuropathy, etc. It is important to note that the prevalence of deficiency states increases with increasing duration of illness, suggesting that they are the result and not the cause of the illness (Stefanini, 1948; Gardner, 1958; O'Brien and England, 1971; Baker and Mathan, 1971). The extent and rate at which these deficiency states develop depend on a number of factors, including the nutritional status of the individual prior to the onset of the illness, the severity of the illness, and the dietary intake during the course of the disease.

Although malabsorption is the major factor in the development of most deficiency states, other factors also play a part. Decreased food intake because of anorexia is common. This anorexia may occur early in the course of the disease (Baker, 1957). As the disease progresses, nutritional deficiencies, particularly of folate and vitamin B_{12}, themselves contribute to the anorexia (Klipstein and Corcino, 1977), forming a vicious cycle. Excessive nutrient losses are obviously important in the fluid and electrolyte disorders; excessive loss of protein (Rubini *et al.*, 1961; Vaish *et al.*, 1965; Jeejeebhoy *et al.*, 1969) and folate (Baker and Mathan, 1971) have also been demonstrated. Finally, decreased synthesis of albumin has been shown to be an important factor in the genesis of hypoalbuminemia in some patients (Jeejeebhoy *et al.*, 1969; Singh *et al.*, 1973).

Examination of the stools usually shows an increase in 24-hr stool volume. Classically, but by no means always, the stools are loose, pale, frothy, and foul smelling. Microscopic examination will usually show fat droplets and at times undigested meat fibers. A careful search should be made for parasites, and if present, their role in the production of the patient's illness must be elucidated by treating the infestation and seeing whether or not the patient's condition improves.

Radiological examination of the gastrointestinal tract is essential to rule out other causes of the malabsorption syndrome. Changes that may be seen in tropical sprue include dilation of the duodenum and small intestine, a coarse mucosal pattern, and alterations in peristaltic activity (Paterson and Baker, 1958). These

abnormalities are more marked with increasing duration and severity of the illness (O'Brien and England, 1971; Baker and Mathan, 1971).

4.3. Absorptive Defects

Although absorption of water and electrolytes (Banwell *et al.*, 1970; Corcino *et al.*, 1973) and many foodstuffs is interfered with, fat, carbohydrate, folic acid, and vitamin B_{12} have been studied the most.

4.3.1. Fat

The proportion of patients found to have steatorrhea varies in different series. In south India (Baker and Mathan 1968b, 1971), north India (Stefanini, 1948; Misra *et al.*, 1967), Singapore (O'Brien and England, 1971), and Puerto Rico (Caldwell *et al.*, 1965), 90–100% of subjects have had steatorrhea. On the other hand, Elder (1947), Gardner (1956), and Jeejeebhoy *et al.* (1966) record steatorrhea in only 50% to 60% of cases. The reason for these differences is not clear. Elder's study was in wartime, before the advent of jejunal biopsy, and it is possible that some of his subjects were suffering from other conditions. Since the degree of steatorrhea tends to be related both to the duration of symptoms and to the severity of the histological changes (Baker and Mathan, 1971; O'Brien and England, 1971), the patients described by Gardner (1956) and by Jeejeebhoy *et al.* (1966) may have been milder cases or earlier in the course of their disease than those studied by other investigators. The prevalence of steatorrhea will also be affected to some extent by the type of fat in the diet, since medium-chain triglycerides are better absorbed than the longer-chain triglycerides (Cancio and Menendes-Corrada, 1964), and unsaturated fats slightly better than saturated ones (Mehta *et al.*, 1971).

Normal fat absorption consists of a number of steps—the formation of micelles, the secretion and action of pancreatic enzymes, the entry of the products of fat digestion into the enterocyte, the resynthesis of triglyceride within the cell, and its transport out of the cell as chylomicrons. It seems probable that in tropical sprue there may be interference with this process at a number of points. Banwell and Gorbach (1969) suggested that bacteria in the jejunum of patients with tropical sprue might cause deconjugation and dehydroxylation of bile salts in the upper small bowel, thus reducing their concentration below the critical micellar level. However, several gropus of investigators have demonstrated that bile salt deconjugation does not occur in the upper intestine (Casells *et al.*, 1970; Kapadia *et al.*, 1971a; Desai *et al.*, 1972; Bevan *et al.*, 1974). On the other hand, Kapadia *et al.* (1971b) found an elevated ratio of glycine to taurine conjugates in the jejunum of sprue patients, suggesting interference with bile salt

reabsorption (McLeod and Wiggins, 1968; Garbutt *et al.*, 1969). This has been confirmed by the finding of excessive loss of labeled bile salts from the body (Kapadia and Baker, 1973) and decreased luminal bile salt concentration in the duodenum (Desai *et al.*, 1972; Bevan *et al.*, 1974). It is probable that these bile salt abnormalities play some part in the pathogenesis of the steatorrhea, but neither the glycine/taurine ratio nor the rate of excretion of bile salts correlates with the degree of steatorrhea, so other factors must also be important.

Exocrine pancreatic secretion following a standard test meal showed a significant reduction in pancreatic function in 24 south Indian patients with tropical sprue as compared with 24 control subjects (Balagopal *et al.*, 1975). However, this study did not enable a differentiation to be made between defective endogenous production of CCK/pancreozymin and an intrinsic defect of pancreatic secretion, nor did it define the precise contribution of pancreatic dysfunction to the fat malabsorption.

In order to throw more light on the relative roles of micelle formation and triglyceride hydrolysis in the steatorrhea of tropical sprue, detailed studies of intraluminal fat digestion will be necessary.

The role of subsequent events in the genesis of the fat malabsorption of tropical sprue has been very inadequately studied. It has been demonstrated *in vitro* that there is both a defect of fatty acid uptake by mucosal cells and a defect of its intracellular conversion to triglyceride, but the mechanisms responsible for this are not clear (Baker and Rao, 1962).

Histochemical studies show an accumulation of lipid in the basement membrane region of the jejunal mucosa in biopsies taken after a 12 to 14-hr fast (Schenk *et al.*, 1965, 1968; Baker and Mathan, 1971). This suggests a holdup in transport of fat enroute from the enterocyte to the lymphatics. Electron microscopy shows that this lipid is, in fact, in a thickened sub-basement-membrane region together with collagen and an amorphous deposit of unknown composition (Baker and Mathan, 1971; Mathan *et al.*, 1975a).

In summary, it is probable that the steatorrhea associated with tropical sprue is multifactorial in origin, but much work remains to be done to fully clarify its pathogenesis.

4.3.2. Carbohydrate

4.3.2a. Glucose. Glucose absorption, as judged by an oral glucose tolerance test, is often impaired in patients with tropical sprue (Fairley, 1936; Stefanini, 1948; Gardner, 1956, 1958; Baker, 1957; Rajan *et al.*, 1961). The precise mechanism of this malabsorption is unknown. Although glucose absorption has been used as a clinical test of intestinal function, the fairly large overlap with normal subjects has caused it to fall into disrepute as a diagnostic test (Gardner, 1956; Rajan *et al.*, 1961).

4.3.2b. Xylose. Xylose absorption has been widely used as a test of intestinal function. The vast majority of investigators have found a high prevalence (73–100%) of xylose malabsorption in subjects with tropical sprue (Klipstein, 1970). The one exception are the patients described by Tandon *et al.* (1966b) of whom only 18% had xylose malasorption. The reasons for the very low prevalence in this study are not clear, but this finding must raise questions about the nature of their cases.

In those regions where tropical enteropathy is common, there will be a high prevalence of xylose malabsorption in the general population, and this must be taken into account in studying the prevalence of xylose malabsorption in patients with sprue. In south India, although there is some overlap, the frequency distribution of xylose excretion in subjects with sprue is different from that of subjects with tropical enteropathy, indicating two distinct populations (Baker and Mathan, 1972). This overlap, however, limits the diagnostic usefulness of the xylose test.

The pathogenesis of the xylose malabsorption is not understood. It is usually assumed to reflect enterocyte damage, but much more work remains to be done to elucidate the precise nature of the defect involved.

4.3.2c. Disaccharides. Several groups of workers have demonstrated that brush border disaccharidase activities are reduced in patients with tropical sprue (Santini *et al.*, 1960; Bayless *et al.*, 1964; Gray *et al.*, 1968; Sheehy and Anderson, 1965; Swaminathan *et al.*, 1970; Corcino *et al.*, 1976).

In view of the fact that the number of mature enterocytes is decreased, and the brush border of individual enterocytes may be sparse and deformed (see Section 4.4), it is to be expected that enzyme activity expressed per milligram of protein or per milligram of tissue will be reduced. It is also not surprising that there is some correlation between the decrease in disaccharidase activity and the degree of histological abnormality (Swaminathan *et al.*, 1970).

4.3.3. Folic Acid

There is a high prevalence of folate deficiency in subjects with tropical sprue (Klipstein, 1972), and it is reasonable to assume that folate malabsorption plays a significant role in the pathogenesis of this deficiency. Surprisingly, tests of folate absorption have provided conflicting results. The situation is further confused by the wide variety of folate absorption tests employed by different investigators (Baker, 1976). Some investigators employing pharmacological doses have demonstrated decreased absorption of pteroylmonoglutamic acid (PGA) in patients with tropical sprue (Butterworth *et al.*, 1957; Chanarin *et al.*, 1958; Jeejeebhoy *et al.*, 1966; Ghitis *et al.*, 1967; Klipstein *et al.*, 1968; Klipstein, 1971). However, in one study, peak serum concentrations following a large

oral dose of PGA were no different in patients with tropical sprue than in control subjects (Jeejeebhoy *et al.*, 1968).

When physiological doses of tritiated PGA are employed in absorption studies, it is more difficult to demonstrate defective absorption. Thus, Jeejeebhoy *et al.* (1967), using a 320-μg dose of PGA, could demonstrate defective absorption in only eight out of 17 patients. In south India, using a 200-μg dose, only nine of 69 patients with tropical sprue had demonstrable malabsorption of PGA (Baker and Mathan, 1971), and using a 25-μg dose Klipstein (1969) found normal absorption in all of three patients with tropical sprue and folate deficience. Smith *et al.* (1970) demonstrated that PGA absorption in the rat is stimulated by the presence of glucose. In man, Gerson *et al.* (1971b) and Corcino *et al.* (1975, 1976) studied PGA absorption from a segment of upper jejunum by the perfusion technique. When the PGA was in glucose- or galactose-free medium, absorption of folate in patients with sprue was not significantly different from normal; however, in the presence of glucose or galactose, the stimulation of PGA absorption was significantly lower in the sprue patients. The biochemical explanation for this phenomenon is not clear, but is is presumably a reflection of mucosal cell damage and may be related to defective absorption of glucose.

The comparative rarity of nutritionally significant PGA malabsorption is further evidenced by the fact that many patients with tropical sprue with folate deficiency megaloblastic anemia will respond to small oral supplements of PGA (Sheehy *et al.*, 1961; Baker and Mathan, 1971). This fact is of considerable practical importance, since it means that it is perfectly feasible to treat the frequently occurring folate deficiency of tropical sprue by the administration of oral folic acid.

The absorption of folate polyglutamates (the major naturally occurring form in foods) is more difficult to study. Hematological methods employing the double reticulocyte response suggested that food folate may be less well absorbed than PGA (Baker, 1966). Other workers, following the rise in serum folate concentration after an oral dose of polyglutamate, demonstrated defective polyglutamate absorption in 50–100% of cases (Jeejeebhoy *et al.*, 1968; Hoffbrand *et al.*, 1969; Bernstein *et al.*, 1970), and Corcino *et al.* (1976), using [^{14}C]hexaglutamate in an intestinal perfusion system, noted improved absorption in six patients with tropical sprue following treatment.

The pathogenesis of folate polyglutamate malabsorption is not clear. It is known that, during absorption, polyglutamates are largely converted to the monoglutamate form (Butterworth *et al.*, 1969). This conversion is catalyzed by the enzyme γ-glutamyl-peptidase (conjugase). Although conjugase is present to some extent in the intestinal lumen, the bulk of it is associated with intracellular lysozymes. Moreover, luminal pH does not favor conjugase activity, and it is probable that the breakdown of polyglutamates occurs mainly within the entero-

cyte. Curiously, measurements of intraluminal (Klipstein, 1967a) and intracellular (Hoffbrand *et al.*, 1969; Corcino *et al.*, 1976) conjugase activity have not shown a significant decrease in the majority of folate-deficient sprue patients. Although the number of mature enterocytes is decreased in patients with tropical sprue, and therefore the total conjugase activity per unit weight of tissue might be expected to be reduced, there is also an increase in number of lysozymes per cell (see Section 4.4.3), which may compensate for the decrease in number of mature cells. In this respect, it would be of interest to compare the relative concentration of conjugase with that of other lysosymal enzymes. Perhaps the normal rate-limiting step is the entry of polyglutamates into the enterocyte—any reduction in the number of mature enterocytes would then result in a reduction of polyglutamate absorption.

4.3.4. Vitamin B_{12}

Vitamin B_{12} malabsorption has been reported to be present in from 17% (Meyer *et al.*, 1953) to 100% (Klipstein *et al.*, 1966a) of patients with tropical sprue. In south India we have observed significant differences in the prevalence of vitamin B_{12} malabsorption between patients with endemic and epidemic sprue and among patients from different epidemics, although the explanation for these differences is obscure (Baker and Mathan, 1971).

The pathogenesis of the vitamin B_{12} absorptive defect is probably multifactorial. It has long been known that patients with tropical sprue frequently have hypochlorhydria or achlorhydria (Fairley, 1930). This is associated with a gastritis which may range from a simple increase in cellularity of the lamina propria to a severe atrophic gastritis or gastric atrophy (Floch *et al.*, 1963; Vaish *et al.*, 1965; Baker, 1967). In 4–7% of cases, intrinsic factor is completely absent, and in a further 7%, it is within the range found in patients with pernicious anemia (Wheby and Bayless, 1968; Baker, 1972). In such cases, if there is no other defect of vitamin B_{12} absorption, repeating the absorption test with exogeneous intrinsic factor will normalize the result (Baker and Rao, 1962). In south India, this only occurred in 10% (3 out of 30) of subjects when the test was repeated with intrinsic factor within 10 days of the original test. In the remainder of cases with vitamin B_{12} malabsorption there must be some other explanation for the defect.

In some patients with tropical sprue vitamin B_{12} absorption may be normalized by the use of broad-spectrum antibiotics (Mollin *et al.*, 1957; Klipstein, 1964a,b; Guerra *et al.*, 1965; Gorbach *et al.*, 1970; O'Brien and England, 1971; Mollin and Booth, 1971; Baker, 1972; Tomkins *et al.*, 1978). In a longitudinal study of 72 south Indian patients with vitamin B_{12} malabsorption, 36 were given antibiotics, and 36 were not—there was no differences in the numbers in the two groups in whom vitamin B_{12} absorption was normalized. However, in those

given antibiotics, vitamin B_{12} absorption often began to improve within 2 to 5 days (Baker, 1967, 1972; Baker and Mathan, 1971).

A similar early response of some patients to antibiotics has been found by Tomkins *et al.* (1978). This rapid response to antibiotics suggests that, in these cases, bacteria must play a role in the pathogenesis of the vitamin B_{12} malabsorption, as has been shown to be the case in the stagnant bowel syndrome (Donaldson, 1962). However, using classical microbiological methods, no quantitative or qualitative difference could be found in luminal microbial flora of jejunum or ileum of patients with and without vitamin B_{12} malabsorption (Bhat *et al.*, 1972). These studies have subsequently been extended to a total of 46 vitamin B_{12} malabsorbers and 40 with normal absorption with similar results (Albert *et al.*, 1982), so it is still not possible to define which bacteria are responsible. Furthermore, studies to try and delineate the mechanism by which bacteria interfere with vitamin B_{12} absorption have been unsuccessful. It has been shown that the intrinsic factor–vitamin B_{12} complex is not taken up or degraded by bacteria and that it reaches the ileum in normal amounts (Kapadia *et al.*, 1975). This suggests that either the intraluminal bacteria produce a "toxin" that damages the receptor or in some other way interferes with the binding of the vitamin B_{12}–intrinsic factor complex to the receptor, or the bacteria responsible for the vitamin B_{12} malabsorption are closely adherent to the mucosal surface and are not sampled by aspiration of luminal contents (Bhat *et al.*, 1980). Carmel *et al.* (1968), in one Puerto Rican patient who may have had tropical sprue, showed defective *in vitro* uptake of vitamin B_{12}–intrinsic factor complex by ileal mucosa, but the mechanism of the defect was not explored. There is obviously a need for further studies of the ileal receptor in patients with sprue to determine the prevalence and pathogenesis of such a lesion.

In those subjects in whom the vitamin B_{12} malabsorption is not improved by antibiotics, it must be assumed that either bacteria play no role in the pathogenesis of the malabsorption or that the bacteria responsible are not susceptible to the antibiotics employed.

4.4. Pathology

4.4.1. Villus Architecture

Even though alterations in villous architecture are common among apparently healthy asymptomatic subjects living in the tropics (see Section 3.2), the abnormalities in subjects with tropical sprue are more severe. However, a "flat mucosa," as seen frequently in cases of celiac disease, is an uncommon occurrence (Baker *et al.*, 1962; England, 1968; Klipstein, 1968; Desai *et al.*, 1969; Brunser *et al.*, 1970; Baker and Mathan, 1971, 1972).

4.4.2. Light Microscopy

Light microscopy reveals changes that vary from "mild," with a slight increase in the depth of the crypts and an increase in the cellular infiltration of the lamina propria and epithelium, to "severe," with reduction in the overall mucosal thickness, crypts extending the full depth of the mucosa, increased numbers of enterocyte mitoses, a cuboidal or pseudostratified appearance of the surface epithelium, and a marked cellular infiltration of the lamina propria and epithelium (Chacko *et al.*, 1961; Baker *et al.*, 1962; England, 1968; Schenk *et al.*, 1965, 1968; Klipstein, 1968; Brunser *et al.*, 1970; Bayless *et al.*, 1971; Baker and Mathan, 1971). The nuclei of the crypt enterocytes may be macrocytic (Veeger *et al.*, 1965; Swanson *et al.*, 1966; Wheby *et al.*, 1968). Although vitamin B_{12} and/or folate deficiency often plays some role in the production of this macrocytosis, it has been observed to persist long after adequate therapy has corrected any vitamin B_{12} or folate deficiency (Wheby *et al.*, 1968), suggesting the presence of some other unknown factor interfering with DNA synthesis in the developing enterocytes. Schenk *et al.* (1965, 1968) describe thickening of the basement membrane region with accumulation of lipid-staining material in this area and suggest that this is characteristic of tropical sprue. We have also seen this in 12 out of 18 subjects with tropical sprue (Baker and Mathan, 1971) but doubt its specificity, since it has also been seen, although to a lesser degree, in asymptomatic control subjects (Mathan *et al.*, 1975a).

Most investigators have shown some correlation between the severity of histological abnormalities and the degree of malabsorption (England and O'Brien, 1966; Kent and Lindenbaum, 1967; Baker and Mathan, 1971; O'Brien and England, 1971; Klipstein, 1971). There is also evidence, at least among expatriates who develop tropical sprue, that the severity of the histological lesion tends to increase with increasing duration of symptoms (England and O'Brien, 1966; O'Brien and England, 1971).

Ileal mucosal biopsies have been studied relatively infrequently. Baker *et al.* (1962) and Wheby *et al.* (1971) found that the histological lesion was generally similar to that seen in the jejunum. England (1968), on the other hand, found that the histological lesion was usually less marked in the ileum. Baker *et al.* (1962) and England (1968) could find no correlation between ileal structure and vitamin B_{12} absorption; however, with larger numbers (27), Wheby *et al.* (1971) did find that the more severe ileal histological abnormalities were related more frequently with the presence of vitamin B_{12} malabsorption and vice versa.

4.4.3. Electron Microscopy

Electron microscopy studies have been reported by three groups of investigators (Hartman *et al.*, 1960; Brunser *et al.*, 1970; Mathan *et al.*, 1975a). One of

these studies was technically unsatisfactory because of poor fixation (Hartman *et al.*, 1960). The other two confirmed the findings on light microscopy and, in addition, found an increase in cytoplasmic lysosomes, shortening and grouping of the microvilli, and deposition of a dense layer below the basement membrane containing collagen, an amorphous material, and fat droplets (Brunser *et al.*, 1970; Baker and Mathan, 1971; Mathan *et al.*, 1975a). The studies in India showed other cellular changes ranging from minor ones, such as dilatation of the rough endoplasmic reticulum and swelling of the mitochondria, to grossly degenerating cells. These were of two types: electron-dense shrunken cells with pyknotic nuclei, increased lysosomes, dilated rough endoplasmic reticulum, and swollen degenerating mitochondria and cells more electron translucent than surrounding cells, with abnormal nuclei, swollen mitochondria, dilated rough endoplasmic reticulum, and distorted microvilli (Mathan *et al.*, 1975a). These dying cells in the crypts and along the sides of the villi strongly suggest that the cells are dying prematurely, presumably damaged by the etiologic agent that causes tropical sprue.

4.5. Therapy

4.5.1. Spontaneous Remission

Unfortunately, there has been insufficient attention paid to the natural history of the sprue syndrome in different parts of the tropics. Spontaneous remission and apparent cure are commonly seen in individuals affected with epidemic sprue in south India (Baker and Mathan, 1968a; Mathan and Baker, 1970). This tendency to remission is less the longer symptoms have been present. Thus, in the first year, 74% remitted spontaneously, whereas of those who had symptoms for more than a year only 8% remitted spontaneously (Mathan and Baker, 1970). A similar tendency to remission has been noted by a number of observers when subjects are hospitalized (Stefanini, 1948; Baker, 1957; Jeejeebhoy *et al.*, 1968; Chuttani *et al.*, 1968a; Gorbach *et al.*, 1970; Baker and Mathan, 1971; O'Brien and England, 1971). This is often ascribed to "rest" or "hosptital diet" but may in fact just be the natural course of the syndrome whether the patient is hospitalized or not. It is of interest that although spontaneous remissions have been described from the Caribbean (Gardner, 1958), it is considered to occur only in "mild" cases (Bayless *et al.*, 1968). This would seem to be at least partly related to the fact that tropical sprue in the Caribbean has long been considered a hematological disorder, and a megaloblastic anemia has been part of the diagnostic criteria. Obviously, subjects with a megaloblastic anemia need to be treated with an appropriate hematinic, and it has therefore not been possible to have a control group that did not receive folic acid or vitamin B_{12}.

4.5.2. Correction of Deficiencies

Correction of deficiency states is obviously important and may at times be life saving, particularly when there are gross fluid and electrolyte disorders (Baker and Mathan, 1971) or severe anemia. Treatment of the anemia used to be dietary, giving yeast, liver, or liver extract (Suarez, 1931; Rhoads and Miller, 1934; Fairley, 1934; Walters, 1947); however, with the isolation of folic acid and vitamin B_{12}, these agents have replaced the dietary management.

4.5.3. Folic Acid

Spies *et al.* (1946) in Cuba were the first to employ folic acid in the treatment of the megaloblastic anemia of tropical sprue. As well as producing a prompt hematological response, it produced a marked decrease in stool volume and a reduction in diarrhea which occurred within 1 to 2 weeks in all except two of nine subjects studied. As this was before the introduction of jejunal biopsy techniques, morphological changes in the intestinal mucosa were not studied. Swanson *et al.* (1966) in Puerto Rico treated seven patients with folic acid. The jejunal biopsy appearances improved in all, but in six out of seven abnormalities persisted. These authors suggest that the folate administration had cured the folate deficiency in the gut but that the basic lesion of sprue persisted. Similar improvement but not cure of the mucosal lesion was seen in the majority of patients treated with folic acid by Sheehy *et al.* (1962b) in Puerto Rico, Klipstein *et al.* (1969) in Haiti, and O'Brien and England (1966, 1971) in Singapore. On the other hand, in Puerto Rico, Rodriguez-Molina *et al.* (1960) and in northern India, Chuttani *et al.* (1968a) were unable to demonstrate any beneficial effect of folate on gut function even though the hematological status was improved. Similarly, in south India, studies of patients with both endemic and epidemic sprue have shown no difference in the remission rate of 57 patients just observed in a metabolic ward and 55 given folic acid (Baker and Mathan, 1971). However, in four patients given folic acid, the stool volume and degree of steatorrhea decreased within a few days, suggesting that in these cases, the folic acid might have been responsible for the improvement.

4.5.4. Vitamin B_{12}

Spies and Suarez (1948), in Puerto Rico, treated four patients with tropical sprue with 10–25 μg of vitamin B_{12} by injection, and in each case the hematological response was accompanied by a decrease in diarrhea. Swanson *et al.* (1966), also in Puerto Rico, found that injections of 30 μg of vitamin B_{12}, daily for 6 days and then weekly, produced improvement in the jejunal biopsy appearances in three of seven patients with tropical sprue. In patients studied in

London with documented vitamin B_{12} deficiency resulting from chronic tropical sprue, the correction of the deficiency sometimes appeared to result not only in a hematological response but also in an improvement in intestinal structure and function (Booth *et al.*, 1968; Mollin and Booth, 1971). In south India, patients with vitamin B_{12} deficiency megaloblastic anemia were treated with vitamin B_{12}, 1 μg/day by injection. A good hematological response was obtained, but there was no detectable effect on intestinal structure or function over and above the similar effect noted in subjects with sprue just given a hospital diet (Baker and Mathan, 1971).

4.5.5. Antimicrobials

The first use of an antimicrobial agent in tropical sprue was by Rogers (1938) who treated a patient suffering from bronchitis with sulfamidochrysoidine (Prontosii®) and noted a coincident symptomatic improvement in his gastrointestinal symptoms. During the 1940s in India sulfaguanidine was found to alleviate the diarrhea of sprue, particularly when given early in the clinical course (Chauduri and Rai Chauduri, 1944; Keele and Bound, 1946; Elder, 1947; Walters, 1947; Stefanini, 1948). Maldonado *et al.* (1969) treated 16 Puerto Rican patients with succinylsulfathiazole or sulfaguanidine for a 6-month period. There was symptomatic improvement in all patients within the first week, and after 6 months, there was marked improvement in intestinal function and histology, although some histological abnormalities persisted. Unfortunately, no controls were used, so it is not possible to be certain that the improvement noted was caused by the sulfonamides.

The use of broad-spectrum antibiotics was introduced by French *et al.* (1956) who studied seven British servicemen repatriated from Hong Kong and Malaya. These workers showed that following the sequential administration of chlortetracycline, chloramphenicol, and succinylsulfathiazole given in various orders, there was rapid clinical improvement and normalization of fat absorption. Although there were no control subjects, there was no significant improvement in the 2- to 4-week period of hospitalization before the start of the chemotherapy, and it seems reasonable to assume that the antibiotics played at least some part in producing the improvement. O'Brien and England (1966, 1971) treated 16 expatriate patients in Singapore with a similar type of regimen. Two of their patients had been ill for less than 3 months and responded rapidly. The other 14 had been ill for 3 months or longer and, in contradistinction to the patients of French *et al.* (1956), "symptomatic improvement was very slow," but there was considerable improvement in intestinal function and structure over the ensuing 1 to 3 months. It must be emphasized that such a slow response is very difficult to interpret because of the marked propensity for patients to undergo spontaneous remission (Baker and Mathan, 1971). In a further group of patients, O'Brien and

England (1971) felt that results of treatment were better when both antimicrobials and folic acid were employed, but these studies were also uncontrolled.

Workers in the Caribbean have also reported favorably on the use of broad-spectrum antibiotics given either for a relatively short period or continuously for up to 6 months (Sheehy and Perez-Santiago, 1961; Guerra *et al.*, 1965; Klipstein *et al.*, 1966a,b, 1969; Horta *et al.*, 1971). However, it must again be noted that in none of these studies were there any controls. Moreover, longer-term follow-up (5 years or more) of a group of 17 patients who had been treated with antibiotics for at least 6 months showed persistence or return of abnormalities of intestinal structure and function in a considerable proportion of patients (steatorrhea in three, abnormal xylose in eight, vitamin B_{12} malabsorption in 13, and abnormal jejunal biopsies in eight) (Rickles *et al.*, 1972). Tomkins *et al* (1975), in London, showed a prompt (within 2 weeks) improvement of intestinal structure and function following a course of tetracycline in four of five patients with acute tropical sprue. In patients with chronic tropical sprue in London (Booth *et al.*, 1968; Mollin and Booth, 1971), the hematological and gastrointestinal abnormalities were "invariably restored to normal" by treatment with folic acid, vitamin B_{12} where necessary, and antibiotics. It should be noted again that there were no controls, and in contradistinction to acute tropical sprue, return to normality often occurred only a number of months after completion of the antibiotic therapy.

In Bombay, Jeejeebhoy *et al.* (1966) obtained marked improvement in absorption tests in the majority of 22 patients with tropical sprue treated with tetracycline for 2 weeks. Gorbach *et al.* (1970), in Calcutta, obtained a marked improvement in steatorrhea and vitamin B_{12} absorption in four out of five patients with sprue within a few days of starting tetracycline therapy. Tandon *et al.* (1974), in Dehli, found improvement in 14 patients treated with a high-protein diet and tetracycline, nine of whom were also given prednisolone; however, histological abnormalities persisted in the jejunum.

On the other hand, Chuttani *et al.* (1968a), also in Dehli, found no response in 73% of subjects treated with sulfonamides, chloramphenicol, or oral streptomycin. Unfortunately, these authors give no breakdown of responses for the three different antibacterial regimens, so it is difficult to assess the significance of their findings. In patients studied in Vellore, antimicrobial therapy also has been less successful. Of 47 patients treated with antimicrobial agents (a sequential combination of tetracycline, chloramphemicol, and succinylsulfathiazole or straight tetracycline) for 2 to 4 weeks, 24 showed no change in steatorrhea; in nine there was improvement within 5 days following the commencement of therapy, whereas in the others the improvement was more gradual, over 10 days to a number of weeks. There was no difference in the folate and vitamin B_{12} status of those who improved and those who did not. This proportion of patients

showing improvement (23/47) is no better than that in a group of 57 patients who were not given any specific therapy other than bed rest and a standard hospital diet, of whom 29 improved (Baker and Mathan, 1971).

4.6. Etiology and Pathogenesis

4.6.1. Introduction

It is evident that the syndrome of tropical sprue is produced by functional and structural abnormalities of the intestine resulting from the action of one or more unknown damaging "agents."

It has been suggested that tropical sprue is the tip of the iceberg of tropical enteropathy (Klipstein, 1967b). Certainly from the clinical and pathological point of view the two conditions seem to grade imperceptibly into each other. However, there are distinct epidemiological differences. Tropical enteropathy is prevalent in areas where sprue does not occur or is comparatively rare, such as Iran (Nasr *et al.*, 1976) and Africa (Cook, 1974a). Also, there are villages in India where tropical enteropathy is prevalent but tropical sprue is absent (Baker and Mathan, 1972). Tropical enteropathy is very common in the first year of life (Chacko *et al.*, 1969), whereas tropical sprue is rare in children and has never been described in the first year of life. Finally, tropical enteropathy improves when subjects return to temperate zones (Sheehy *et al.*, 1968), whereas sprue may develop years after leaving the tropics. We therefore believe that these are two independent conditions of differing pathogenesis. Field studies in south India have shown that not every agent producing intestinal damage results in the sprue syndrome (Mathan and Baker, 1968); there is therefore at least some specificity in the unknown initiating agent or agents. It must also be borne in mind that the effects of any initiating agent may be modified by the interplay of various aggravating or perpetuating factors (Baker and Mathan, 1968a).

Any comprehensive explanation of the sprue syndrome must account, *inter alia,* for a number of distinctive aspects of the condition such as:

1. Its geographical distribution.
2. Its occurrence in epidemic form.
3. The long latent period which may sometimes occur between leaving the tropics and developing the disease.
4. The pattern of therapeutic responses to folic acid, vitamin B_{12}, and antibotics.

Possible etiological factors are nutritional deficiencies, dietary factors, or infectious agents (parasites, algae, fungi, bacteria, mycoplasma, or viruses).

4.6.2. Nutritional Deficiency

Because of the frequent occurrence of deficiency states in patients with advanced tropical sprue, it is perhaps inevitable that deficiences should be postulated as one cause of this syndrome (Walters, 1947; Stefanini, 1948; Ayrey, 1948). A deficiency state *per se* will not explain the geographical distribution of the condition, but it could perhaps be combined with some other factor with a limited geographical distribution. Although it might be thought that the occurrence of epidemics precludes a deficiency state, it must be recalled that epidemics of deficiency disease are well documented, e.g., pellagra (Goldberger *et al.*, 1920). However, it is hard to visualize how a deficiency state could explain the very long latent period that may occasionally occur.

4.6.2a. Folate. The sometimes dramatic response of patients with sprue to folic acid has led to the postulate that the syndrome may be caused by dietary folate deficiency (Spies *et al.*, 1946). Although many patients with tropical sprue are folate deficient, the prevalence of folate deficiency increases with increasing duration of symptoms (Baker and Mathan, 1971; O'Brien and England, 1971), suggesting that it is a result and not the cause of the syndrome. Moreover, subjects with folate deficiency megaloblastic anemia in Nairobi (Foy and Kondi, 1971) and in south India, where tropical sprue is highly prevalent (Baker and Mathan, 1971), do not develop the syndrome of tropical sprue. Furthermore, dietary deficiency of folate has usually been reported as producing no change in the appearance of the jejunal mucosa (Herbert, 1962; Gough *et al.*, 1963; Forshaw *et al.*, 1964; Halstead *et al.*, 1973) or, in the presence of alcoholism, minor changes (Bianchi, 1970; Hermos *et al.*, 1972). It must be concluded, therefore, that there is no evidence that folate deficiency is the cause of tropical sprue but that folate deficiency is common and frequently contributes to the symptom complex, most notably to the anemia but also at times to the gastrointestinal dysfunction. The greater the contribution to the gastrointestinal lesion, the more dramatic will be the response to folate therapy in improving gastrointestinal function. For some as yet unexplained reason folate deficiency seems to play a smaller role in the gastrointestinal lesion of patients in India than in patients from other areas (see Section 4.5.3).

4.6.2b. Vitamin B_{12}. Although the administration of vitamin B_{12} to patients with sprue may, at times, improve not only the hematological status but also in some cases the gastrointestinal function (see Section 4.5.4), vitamin B_{12} deficiency in nonvegetarian subjects usually only develops months or years after the onset of the disease (O'Brien and England, 1971; Mollin and Booth, 1971). Vitamin B_{12} deficiency may therefore be excluded as a primary cause of the

syndrome, although, like folate deficiency, it may at times be an aggravating or perpetuating factor.

4.6.2c. Protein. The administration of a protein-deficient diet to animals has produced histological changes in the jejunal mucosa in some studies (Platt *et al.*, 1964; Deo and Ramalingaswami, 1965; Mehta *et al.*, 1979) but not in others (Svoboda *et al.*, 1966; Tandon *et al.*, 1969). These differences may be related to the different species employed and to differing degrees of protein restriction (Baker, 1977). The applicability of these studies to the human situation is very doubtful, since the amounts of protein used were much lower than those habitually consumed in areas where sprue is endemic. Nevertheless, it has been suggested by several workers that protein deficiency may produce changes in intestinal structure and function in adults and be a cause of the tropical sprue syndrome (Mayoral *et al.*, 1967, 1968, 1972; Chuttani *et al.*, 1968b; Tandon *et al.*, 1968; Duque *et al.*, 1975). Unfortunately, all of these studies were conducted in areas where tropical sprue and other forms of intestinal disease are prevalent, and it is impossible to be sure whether the hypoproteinemia caused the gut disease or vice versa.

In patients with tropical sprue, the prevalence and severity of hypoproteinemia increases with increasing duration of the illness (Baker and Mathan, 1971; O'Brien and England, 1971), suggesting that the hypoproteinemia is a result of the disease. Patients living in a temperate climate who have hypoproteinemia do not have mucosal abnormalities (Gough *et al.*, 1963; Jensen *et al.*, 1966; Marin *et al.*, 1969), and three Zambian adults with severe hypoproteinemia were shown to have normal intestinal structure and function (Cook, 1974b). It must be concluded that there is inadequate evidence to consider deficient protein intake as a cause of tropical sprue, although at times, like folate and viatmin B_{12} deficiency, protein deficiency may act as an aggravating or perpetuating factor.

4.6.3. Dietary Factors

French (1955) suggested that consumption of rancid fats might be a cause of tropical sprue. However, there is no evidence to suport this hypothesis, and it does not explain why it occurs in some parts of the tropics and not in others. Moreover, it is hard to see how the ingestion of a dietary toxin can explain the phenomenon of the long latent period.

Klipstein and Corcino (1974) noted in Puerto Rico that the peak seasonal incidence of tropical sprue coincided with the time of maximum consumption of pork and pork fat. Mickelson and Klipstein (1975) postulate that the long-chain unsaturated fatty acids, particularly linoleic, have an inhibitary effect on the

gram-positive intestinal bacteria, allowing overgrowth of coliforms which, in turn, produce enterotoxins which cause disease (see Section 4.6.4). This is an interesting hypothesis which needs to be further explored. It would not, however, seem to be relevant in south India where the dietary fat intake is very low and where there is no clear-cut seasonal pattern.

Although gluten-sensitive enteropathy occurs in regions of the tropics where wheat is eaten, it seems to be of low prevalence (Misra *et al.*, 1966; Walia *et al.*, 1966; Nelson, 1973). Bayless (1964) found some improvement in the intestinal lesion of some patients with sprue when they were put on a gluten-free diet, but this was probably a nonspecific effect. Subjects with tropical sprue do not have gliadin antibodies in their serum (Heiner *et al.*, 1964; Bayless *et al.*, 1967; Menéndez-Corrada and Belaval, 1968), and tropical sprue is prevalent in non-wheat-eating populations (Baker *et al.*, 1963). It therefore seems unlikely that sensitivity to gluten plays any significant role in the pathogenesis of the syndrome.

4.6.4. Infectious Agents

An infectious agent has long been postulated as the cause of tropical sprue (Grant, 1854; Galloway, 1905a,b; Begg, 1912). Such a hypothesis could explain the geographical distribution of the disease, the occurrence of epidemics, and, conceivably, the long latent period.

4.6.4a. Parasites. In the past, certain parasitic diseases of the intestine have doubtless been labeled "tropical sprue" (e.g., giardiasis, strongyloidiasis). However, patients whose malasborption is caused by these organisms will respond to appropriate therapy and do not come within the definition of the tropical sprue syndrome. If the patient's condition does not improve after erradication of the parasite, then it can be assumed that there is some complicating factor or that the disease is not caused by that parasite.

4.6.4b. Algae. There is one report, which has appeared only in abstract form, suggesting that the alga *Prototheca portoricensis* might be the cause of tropical sprue (Bernstein *et al.*, 1973). However, this organism has never been isolated from patients with sprue or visualized in biopsy specimens (Bhat *et al.*, 1972; Klipstein and Schenk, 1975b; Corcino, 1975) even though it has been looked for and is easy to culture. This hypothesis must therefore be abandoned.

4.6.4c. Fungi. Bahr (1915) and Ashford (1931) postulated that sprue was an infection caused by *Monilia ablicans,* but subsequent studies specifically looking for fungi have failed to confirm this suggestion (Milanes *et al.*, 1946; Bhat *et al.*, 1972).

4.6.4d. Bacteria. Bacteria may cause intestinal disease either by invasion of the mucosa (e.g., Whipple's disease) or by the production of enterotoxins. Light and electron microscopic studies of jejunal biopsies from patients with sprue have consistently failed to reveal any evidence of bacteria in the mucosa, so that bacterial invasion as a cause of the syndrome can be excluded (Hartman *et al.*, 1960; Chacko *et al.*, 1961; Baker *et al.*, 1962; Swanson *et al.*, 1966; England, 1968; Klipstein, 1968; Wheby *et al.*, 1968; Brunser *et al.*, 1970; Mathan *et al.*, 1975a).

Many investigators have unsuccessfully cultured stools and/or luminal aspirates for classical bacterial pathogens in patients with sprue (Nadel and Gardner, 1956; Desai *et al.*, 1966; Klipstein and Samloff, 1966; O'Brien, 1968; Baker and Mathan, 1971; Gorbach *et al.*, 1969, 1970; Bhat *et al.*, 1972). Similar negative results were obtained from culture of jejunal biopsy material (Bhat *et al.*, 1980). Even in epidemics of sprue, known pathogenic organisms have not been isolated with any greater frequency than in control subjects (Baker *et al.*, 1963; Mathan *et al.*, 1966; Baker and Mathan, 1971).

Although classical pathogenic organisms have not been incriminated, a number of investigators in different parts of the world have demonstrated the presence of increased numbers of luminal bacteria in the small intestine in many subjects with tropical sprue (Milanes *et al.*, 1946; Nadel and Gardner, 1956; Klipstein *et al.*, 1966a, 1973b; Lahri *et al.*, 1970; Gorbach *et al.*, 1969, 1970; Bhat *et al.*, 1972; Tomkins *et al.*, 1975; Applebaum *et al.*, 1980). In a number of these studies, at least some of the control subjects also had an abnormal bacterial flora, and in several studies, no difference could be detected between the patients with sprue and the control subjects with respect to the types of organism and their prevalence and distribution within the gastrointestinal tract (Lahri *et al.*, 1970; Bhat *et al.*, 1972, 1980; Applebaum *et al.*, 1980). It is noteworthy that in the studies of Bhat *et al.* (1972, 1980) and Applebaum *et al.* (1980), there was a mixed aerobic and anaerobic flora, whereas in the studies of Klipstein *et al.*, 1966a, 1973a), Gorbach *et al.* (1970), and Tomkins *et al.* (1975), although none of the papers give full bacteriological details, the predominant organisms appear to have been coliforms.

The mechanisms that determine the type and extent of the microbial flora in the small intestine are not well understood. Factors of importance are probably the bacterial load ingested, the nature of the diet (Klipstein and Corcino, 1974), the gastric pH, the secretory immune system of the gut, the flushing action of the flow of luminal contents, and the differing abilities of organisms to adhere to the mucosa. These factors have received very little attention. Cook (1978b) postulates that decrease in intestinal motility is the cause of the bacterial overgrowth in tropical sprue. This hypothesis, however, ignores the fact tht although small intestinal transit time is prolonged in many cases of sprue, in others transit time is shortened (Baker, 1957; Paterson and Baker, 1958; Baker and Mathan, 1971;

Tomkins *et al.*, 1975), and in both situations bacterial overgrowth may be present. Moreover, it does not explain the bacterial overgrowth seen in many healthy control subjects.

The precise role of intestinal bacteria in the pathogenesis of tropical sprue is not clear. It should be noted that not all subjects with tropical sprue have abnormal numbers of bacteria in the jejunum (Nadel and Gardner, 1956; Lahri *et al.*, 1970; O'Brien and England, 1971; Bhat *et al.*, 1972, 1980), nor do all subjects with an abnormal flora have malabsorption (Lahri *et al.*, 1970; Gorbach *et al.*, 1970; Bhat *et al.*, 1972). However, the observed responses to antimicrobial therapy (see Section 4.5.5) suggest that bacterial overgrowth may play an important role in some cases.

To further elucidate this problem, Klipstein and his colleagues have undertaken extensive studies of toxin production by organisms isolated from patients with tropical sprue. They found that coliforms (*Klebsiella pneumoniae, Enterobacter cloacae,* and *Escherichia coli*) isolated from the midjejunum of Puerto Rican patients with tropical sprue produced a toxin that caused fluid secretion when injected into rabbit ileal loop preparations (Klipstein *et al.*, 1973b). This toxin also produced histological changes in the rabbit loops, and it was suggested that it might be responsible for the histological changes seen in the jejunal mucosa of patients with tropical sprue (Klipstein and Schenk, 1975a). The toxin has been shown to be heat stable, to resist acid treatment, and to have a molecular weight in the region of 1000–10,000 (Klipstein and Engert, 1975). When the toxin was perfused in rat small intestine, net secretion of water and sodium resulted (Klipstein *et al.*, 1975). Similar toxigenic coliforms were isolated from four of five Haitian patients with sprue but not from those with tropical enteropathy (Klipstein *et al.*, 1976). In a further extension of this work, Klipstein *et al.* (1978) compared 12 patients with tropical sprue (nine from Puerto Rico and three from Haiti) with five patients with blind loop syndrome from Rochester, New York. Fourteen out of 16 strains of coliforms isolated from patients with sprue produced heat-stable and/or heat-labile toxins. These produced net water secretion in the rat model in the same concentration range as that at which the toxin of classical toxigenic strains of *Escherichia coli* (from patients with acute gastroenteritis) was active. On the other hand, coliform strains isolated from the patients with the blind loop syndrome, and those isolated from healthy individuals, only produced toxin effective in at least 1000-fold higher concentration. These authors suggest that the malabsorption and histological changes in tropical sprue may at least partly be accounted for by the presence of the toxin-producing organisms.

On the other hand, Albert (1978), also using the rabbit ileal loop technique, studied isolates from 12 south Indian patients with tropical sprue. In only three of the 12 patients were toxin-producing coliforms found in the jejunum (four out of 23 strains studied), and in only two out of seven patients sampled were toxin-

producing organisms found in the ileum (three out of 16 strains studied). It is clear that the bacterial flora in these patients are very different from those of the Caribbean patients described by Klipstein *et al.* (1973b). This lower prevalence of toxin-producing organisms may account for some of the differences seen in response to antibiotic treatment in the two areas (see Section 4.5.5). Further, if one cause of the sprue syndrome is overgrowth with toxin-producing organisms, then at least many of the south Indian cases would appear to be of a different etiology.

4.6.4e. Mycoplasma. Lev *et al.* (1969) studied jejunal aspirates and jejunal biopsies from patients with tropical sprue and obtained mycoplasma from six of 12 patients with sprue and two of 12 control subjects. Unfortunately, the organisms were not typed, and no follow-up work has been published by these investigators. In south India no mycoplasma could be recovered from jejunal secretions or jejunal biopsies of 23 patients with sprue or 28 controls, although 45% of all salivary specimens grew mycoplasma, indicating that the methodology was adequate (Bhat *et al.*, 1973). It must be concluded that there is presently no evidence to incriminate any mycoplasma as a cause of the sprue syndrome.

4.6.4f. Viruses. Manson-Bahr (1957) suggested that tropical sprue might be an infection with a virus "like the herpes virus, which can maintain itself unchanged for many years in the human body. . . ." A viral etiology could explain many of the peculiar features of tropical sprue including the long latent period, the occurrence of epidemics, the altered age-specific attack rate in second epidemic waves, the clustering of cases in space and time seen in epidemics, and the occurrence of fever at the onset of illness in a proportion of cases. Although most viral diseases are not limited by geographical boundaries, a vector-borne virus could well explain the peculiar geographical distribution of the disease (e.g., yellow fever). In this respect, it is of interest that in one village epidemic of sprue in south India, the attack rate correlated with the amount of time spent in the house and with the prevalence of bedbugs in the house (S. J. Baker and V. I. Mathan, unpublished observations, 1970), suggesting a possible vector.

A number of epidemics of gastroenteritis have been caused by viruses (Reimann, 1963; Estes and Graham, 1979). The two most studied viral agents are the 27-nm diameter Norwalk Agent and the 70-nm rotavirus (Kapikain *et al.*, 1972, 1978; Bishop *et al.*, 1973). However, these agents cause a short-lived illness from which recovery is usually complete with little evidence of persisting malabsorption. Attempts in Puerto Rico (Bayless *et al.*, 1966) and south India (Baker, 1971) to grow viruses from patients with tropical sprue have been unsuccessful.

In 1975, we found coronaviruslike particles in the stools of south Indian control subjects and patients with tropical sprue (Mathan *et al.*, 1975b). Because

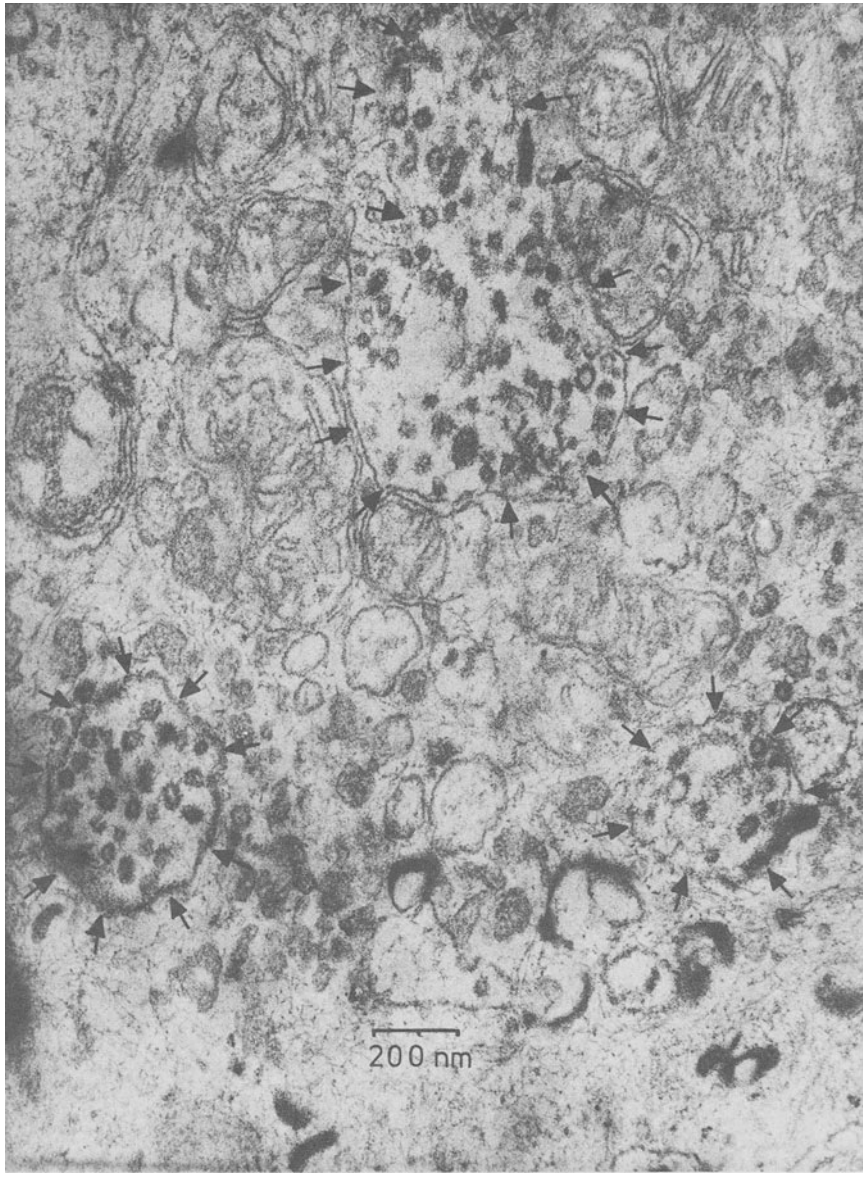

Figure 1. Electron micrograph of dying enterocyte from crypt area of an Indian patient with intestinal malabsorption and a gastroenterostomy, showing coronaviruslike vesicles (arrows).

of its occurrence in subjects without sprue, we tended to dismiss it as being of no significance. However, it is clear that in the case of many viruses, only a proportion of infected individuals develop clinical illness. It is therefore of interest that more recently we have observed vesicles containing coronaviruslike particles (Figure 1) in the pale-staining dying enterocytes of jejunal biopsies from a south Indian man with intestinal malabsorption who had also had a gastroenterostomy (Baker *et al.*, 1982). It was not possible to be sure how much of his malabsoprtion was caused by the mucosal disease and how much was caused by the gastroenterostomy. However, review of previously taken electron micrographs showed similar virus particles in pale-staining dying enterocytes in three of 12 patients with chronic tropical sprue, suggesting that this virus was either an opportunistic one, colonizing dying cells, or else, as seems more probable, it was causing premature enterocyte death. This virus must therefore be considered a serious candidate as one possible etiologic agent producing the intestinal damage that causes tropical sprue.

4.7. Summary and Conclusions

The rather confusing picture of the tropical sprue syndrome that emerges at the present time may perhaps be simplified by considering the pathogenesis in at least two steps, namely, the first step responsible for initiation of intestinal damage and the second which perpetuates or aggravates it.

The varying reports of response to treatment with folic acid, vitamin B_{12}, and antimicrobials are undoubtedly, in part, related to the total lack of controlled therapeutic trials. Nevertheless, when rapid improvement in intestinal function and structure occurs following institution of a given therapy, it is reasonable to assume that the administered therapy may have played some part in producing the improvement. On this basis, it appears that in certain patients with tropical sprue, folate and/or vitamin B_{12} deficiency may be perpetuating or aggravating the intestinal damage, even though there is good reason to believe that they are not initiating factors. Protein deficiency may play a similar role. In a larger group of patients, the reported responses to antimicrobial therapy indicate that bacterial overgrowth, perhaps particularly, overgrowth with enterotoxigenic bacteria, may be a perpetuating or aggravating factor. Clearly, also, these factors may act alone or in combination with each other, so that treatment with one or any combination of folate, vitamin B_{12}, protein, and antimicrobials may be necessary to produce improvement.

The nature of the initiating agent(s) is less clear. Deficiency states can probably be eliminated for reasons given above. Studies from Klipstein's group suggest, but have not proven, that colonization with enterotoxogenic bacteria may also produce the initial damage (Klipstein, 1979). If this be so, since bacteria are ubiquitous, some other factor would have to be involved to explain

the unusual geographic distribution of the syndrome, such as a dietary pecularity (Klipstein and Corcino, 1974). However, it is clear from studies in Vellore that many south Indian subjects with tropical sprue do not have toxin-producing organisms in their intestinal tract. In these cases, some other initiating agent(s) must be responsible. We believe, but have not yet proved, that at least one candidate agent is a coronaviruslike infection of the enterocytes. Thus, on the basis of available evidence, the hypothesis is advanced that there are at least two major forms of the syndrome of tropical sprue (Figure 2), viz:

1. Bacterial-initiated sprue (BIS), where colonization of the upper intestine with toxigenic bacteria plays a dominant role in initiation and, because of their continuing presence, the perpetuation of the disease.
2. Viral-initiated sprue (VIS), where the initiating agent is a coronaviruslike (or other viral) infection of the enterocytes which may, at times, also be complicated by secondary bacterial overgrowth.

Either form may be further complicated by folate or vitamin B_{12} and perhaps protein deficiency.

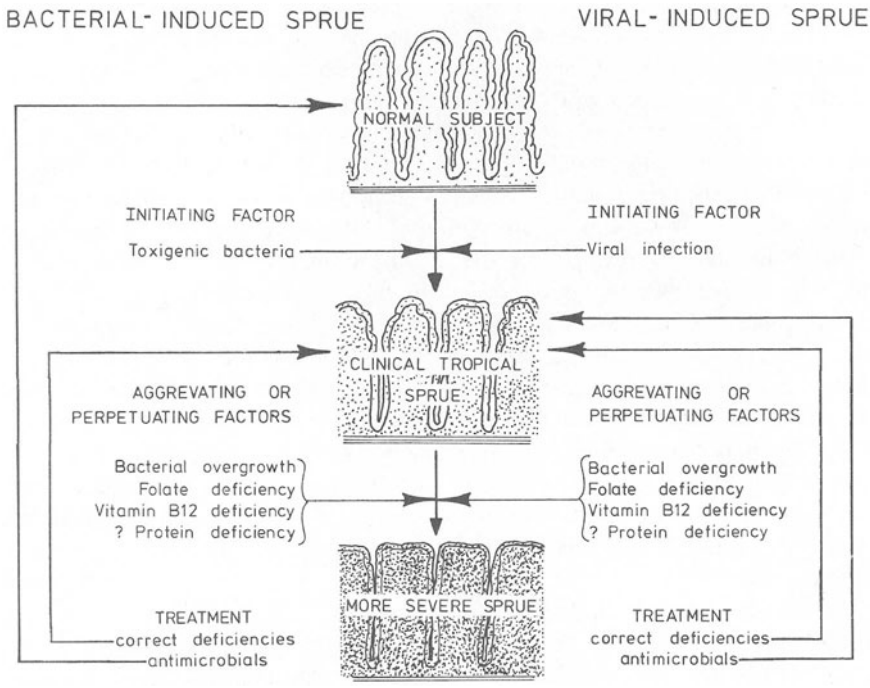

Figure 2. Diagrammatic representation of the suggested pathogenesis and response to treatment of two forms of tropical sprue—bacterial induced sprue (BIS) and viral induced sprue (VIS).

This hypothesis explains the variable responses to folate and vitamin B_{12} supplementation [e.g., in cases where folate deficiency is exerting a major influence on intestinal function, folate therapy will have a marked effect (Spies *et al.*, 1946), but where it has only a minor effect or is absent, folate therapy will produce little or no change]. It also explains the apparent cure with antimicrobial therapy of many cases of tropical sprue (BIS) (e.g., in the Caribbean) and the failure of the same therapy in other cases (VIS) (e.g., in south India). Viral initiated sprue is probably responsible for the epidemics of sprue and the form of the disease with a long latent period. The few reports of "tropical sprue" in temperate zones may be cases of BIS syndrome where, for some reason, toxigenic coliforms have colonized the upper intestine.

Future research must be aimed at clarifying a number of points including the following:

1. The factors that control the microecology of the upper small intestine.
2. Whether BIS is indeed initiated by the bacteria themselves or whether some other initiating agent is involved and the bacterial overgrowth is only a secondary phenomenon.
3. The precise factors that determine the geographical distribution of BIS.
4. The fulfilling of Koch's postulates with regard to the role of the coronaviruslike agents, or other agents, in the initiation of VIS.
5. The mode(s) of transmission of VIS and its true geographical distribution.

When answers to these questions are available, we will understand much more about this baffling but fascinating syndrome and be in a better position to rationally treat and prevent it.

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