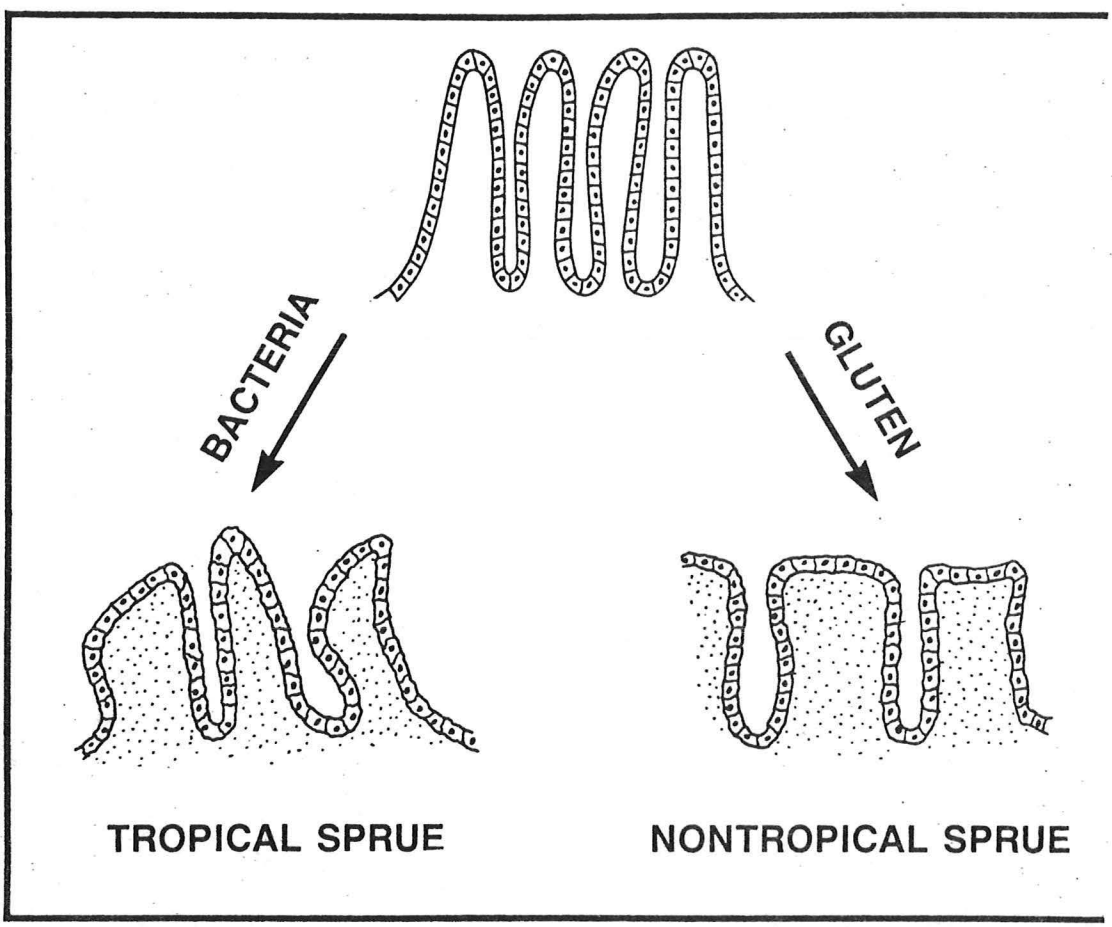
Castro

THE SPRUE SYNDROMES



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Sprue as a disease entity was first described a century ago, but the distinction between nontropical and tropical sprue was not recognized until recently, and the two diseases were regarded as one disease that could be acquired both in the tropics and in a temperate climate. The two diseases share common features in that they both present with a malabsorption syndrome and villus atrophy of the small intestine. Nontropical and tropical sprue are, however, distinctly different with respect to etiology, pathogenesis and treatment. The purpose of this review is to examine the more recent knowledge of the two diseases with a focus on the cell kinetics of the mucosal lesion, the etiology and pathogenesis, and the pathophysiology of the malabsorption syndrome.

Background

The unusual name "sprue" was introduced into the English language by Sir Patrick Manson in 1880 (1) by an anglicization of the Dutch name "Spruw" which means thrush. While residing in China, Manson saw many cases of what was called "indische spruw" characterized by oral aphthae associated with chronic diarrhea and wasting. He considered sprue to be a specific disease entity unrelated to parasitic infestations and noted that it was "exceedingly insidious in onset and very slow in progress" but often of a long duration and with a substantial mortality. Within the same decade, "celiac disease" was first described by Samuel Gee, a physician at St. Bartholomew's Hospital in London in a short paper, "On the Coeliac Affection" (2) where he described his personal experience of cases of celiac disease. In this paper he makes several pertinent observations which are still valid today. He noted that the disease was common in childhood and that "signs of the disease are yielded by the feces," which he described in considerable detail, and that the disease was characterized by chronic diarrhea, wasting and failure to thrive. He also noted that "sometimes from India Englishmen return sick with the coeliac affection" which now obviously were cases of tropical sprue. The cause of celiac disease was obscure and autopsy studies did not reveal any abnormality of the intestines or other digestive organs. Further, he makes the prophetic statements that "the allowance of farinaceous food must be small" and that "if the patient can be cured at all, it must be by means of diet."

Over the next forty years many case reports appeared on the sprue syndromes and the name tropical sprue was reserved for cases contracted in the tropics whereas the nontropical variety was called by many names such as celiac disease, Gee-Herter's disease, intestinal infantilism and idiopathic steatorrhea (3-5). It was also recognized that the hallmark of both diseases was fat malabsorption and that diet may play a role in the treatment of nontropical sprue. It was not recognized, however, that the two diseases were distinct entities, and in a review in 1939 Snell (6) concluded that "if sprue is sought for as vigorously in Northern climates as it is in the tropics, it seems certain that the artificial distinctions which have been set up between tropical and nontropical sprue will soon be regarded as unimportant." Surprisingly, this view persisted up untill about 1960 when Green and Wollaeger from the Mayo Clinic endorsed the concept that the two diseases were identical (7), although some significant advances in the treatment of tropical and nontropical sprue had been made. In the 1930s the treatment of tropical sprue was successfully achieved with folic acid which improved both the

hematologic abnormalities and the malabsorption. During the second World War the British used oral sulfonamides in the treatment of tropical sprue among their troops in India and obtained symptomatic improvement in most cases (8). The combination of folic acid and now tetracycline has since become the most effective treatment of tropical sprue. Furthermore, in 1950, Dicke (9) described the dramatic effect of a gluten-free diet in nontropical sprue with reversal of the devastating symptoms.

The cause of the malabsorption in the sprue syndromes had eluded all investigators despite numerous autopsy studies of fatal cases mainly due to the autolytic changes which set in rapidly in the small intestine. Schein probably should be credited with the first description of villus atrophy in a boy who died at age 15 from nontropical sprue (10). The autopsy was carried out shortly after death, and all tissues were immediately fixed in formalin and histological examination of the small intestine showed that "the villi were broad based, squat, bulbous and plump." The findings were confirmed by the same author within the same year in autopsy studies of another six patients with nontropical sprue (11). In 1954, Paulley reconfirmed this finding in four living patients with nontropical sprue who underwent laparotomy with small bowel biopsies (12). Histological examination demonstrated a flat mucosa with villus atrophy and dense inflammation of the lamina propria. A similar change in the morphology of the small intestinal mucosa was also later demonstrated in tropical sprue (13). In general, the mucosal changes in tropical sprue are not as marked as in nontropical sprue. Since the introduction of the peroral small intestinal suction biopsy in the 1960s, the mucosal changes in tropical and nontropical sprue have been demonstrated in multiple reports on these diseases, and it is now well recognized that similar changes, i.e. villus atrophy, are characteristic for both. Also, during the same decade, it was finally settled that the two diseases were distinct entities with different etiology, pathogenesis and treatment. The current definitions and characteristics of nontropical and tropical sprue are outlined in Table 1.

NON-TROPICAL SPRUE

- Villus Atrophy
- Malabsorption
- Progressive if Untreated
- Highest Incidence in Temperate Climates
- Responds to Gluten-free Diet
- Clinical and Morphological Deterioration on Rechallenge with Gluten

TROPICAL SPRUE

- Villus Atrophy
- Malabsorption
- Progressive if Untreated
- Contracted in the Tropics
- Responds to Folic Acid and Tetracycline

TABLE 1: *The characteristics of nontropical and tropical sprue*

Small Intestinal Morphology in the Sprue Syndromes

The normal small intestinal mucosa is characterized by being a highly folded membrane due to presence of the intestinal villi which are tall, slender, finger-like structures as illustrated in Figure 1.

The intestinal villus consists of a central core which contains a capillary network and the central lacteals. The core is covered by a layer of columnar epithelial cells interspersed with mucin-producing goblet cells and endocrine cells. At the base of the villus is the crypt zone or the germinative epithelium from which the new cells are formed. The intestinal mucosa has the most rapid turnover of any tissue in the body. The newly formed cells in the crypts are pushed out on to the side of the villus, and over the course of 3 to 5 days move to the tips of the villi where they die and are shedded off into the intestinal lumen. In other words, each columnar epithelial cell has a short and finite life span. Doniach and Shiner (14) compared the intestinal mucosa with the bone marrow and suggested that the mature absorbing cells, which do not divide and have a limited life span, are analogous to peripheral blood cells. Booth (15) extended this analogy and suggested that the crypt cells should be called "enteroblasts" and the mature absorbing cells "enterocytes," and the whole process of cell turnover could be termed "enteropoiesis." Each enterocyte is hexagonal in shape, attached to the basal membrane at the basal borders of the cell and attached to neighbor

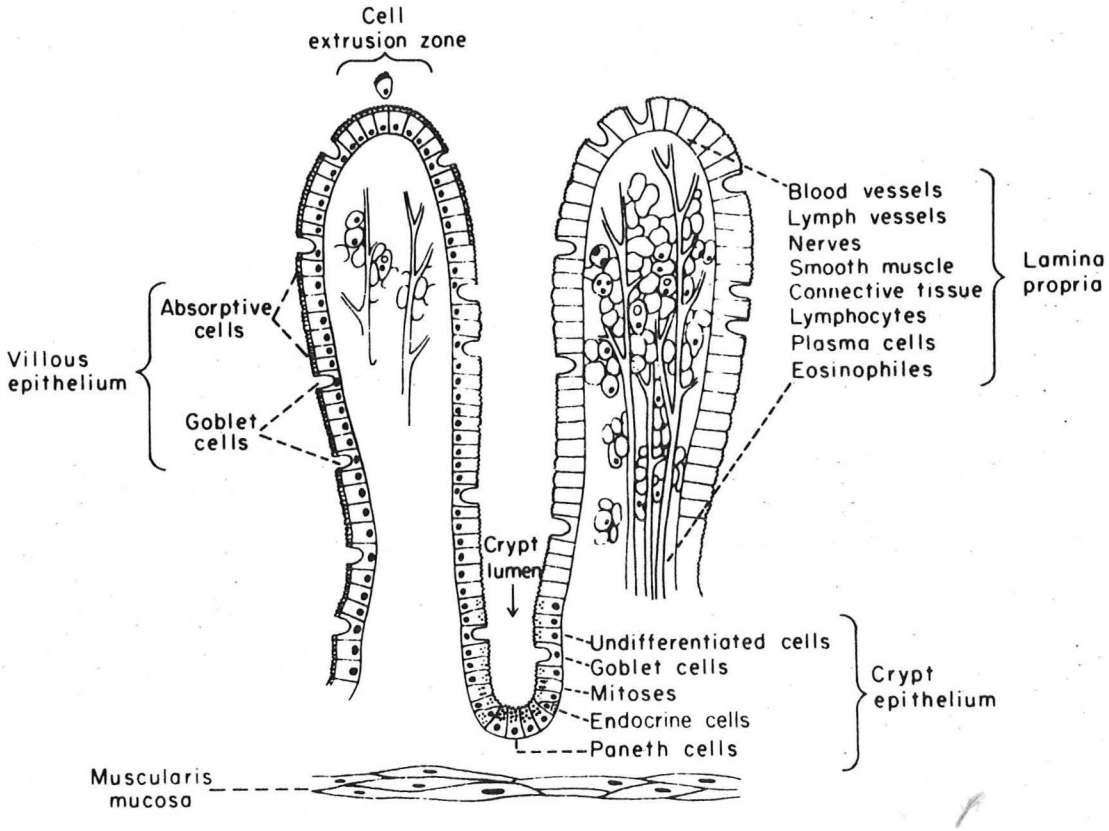

FIGURE 1: *Anatomy of the Villus*

cells with tight junctions. The luminal surface of the enterocyte is covered by microvilli about 1 μm in height, and the microvilli are covered by a "fuzzy coat" or glycocalyx, which presumably serves as a protective coat for the microvillus membrane.

In contrast, the intestinal mucosa in the sprue syndromes is distinctly different. When the mucosa is viewed at low power through a dissecting microscope, it appears flat with lack of discernible villus structures. At higher power the mucosa has a mosaic pattern and the crypts can be easily seen to open on to the surface of the mucosa. Histological examination of the flat mucosa reveals three distinct features apart from the loss of the villi. First, the epithelial cells are no longer columnar but flat, cuboidal with a reduced number. Second, the crypts are enlarged both in length and in width with an increased number of enteroblasts. Third, the lamina propria is infiltrated with plasma cells and lymphocytes.

There are two possible mechanisms which can account for the distinct changes of the intestinal mucosa in the sprue syndromes and produce villus atrophy. There may be direct damage of the enterocytes leading to increased number of dead enterocytes per unit time and the crypt hyperplasia is a compensatory reaction to increase the production of enterocytes analogous to hemolytic anemia (14). Or the maturation of enteroblasts to enterocytes may be inhibited and the production rate of enterocytes therefore reduced by maturation arrest, a situation which can be compared to the hyperplastic bone marrow seen in pernicious anemia.

Booth reviewed the evidence for the two hypotheses in 1970 (15), and favored the first although definite proof for either hypothesis was limited, and his contention was based mainly on the fact that small intestinal perfusion studies had demonstrated an increased amount of DNA in the perfusate in patients with celiac disease as compared with controls (16). The increased amount of DNA was thought to be derived from the desquamated enterocytes and thus reflects increased cell death.

The question whether the flat mucosa was a result of increased cell death with compensatory crypt cell hyperplasia or the result of maturation arrest remained unsettled and over the last 15 years there has been much interest in the description of the cell kinetics of the small intestinal mucosa both in normals and in various disease conditions such as the sprue syndromes. A variety of techniques have been utilized such as incorporation of ^{3}H-thymidine, estimation of mucosal DNA content, sthatmokinetic studies and morphometric measurements (17-22). There are various pitfalls in each of these methods, and the conclusions derived from many of these studies should be viewed with caution (23). The mucosa of the small intestine has a complex three-dimensional structure, and the quantitative morphology is exceedingly difficult to study as a conventional histological section only represents a two-dimensional view.

The purpose of cell kinetic studies is to define the proliferative status of the intestinal epithelium and thus to examine whether cell proliferation is increased or decreased in a particular disease. The cell kinetics in the small intestinal mucosa is determined by the parameters outlined in Figure 2 (23).

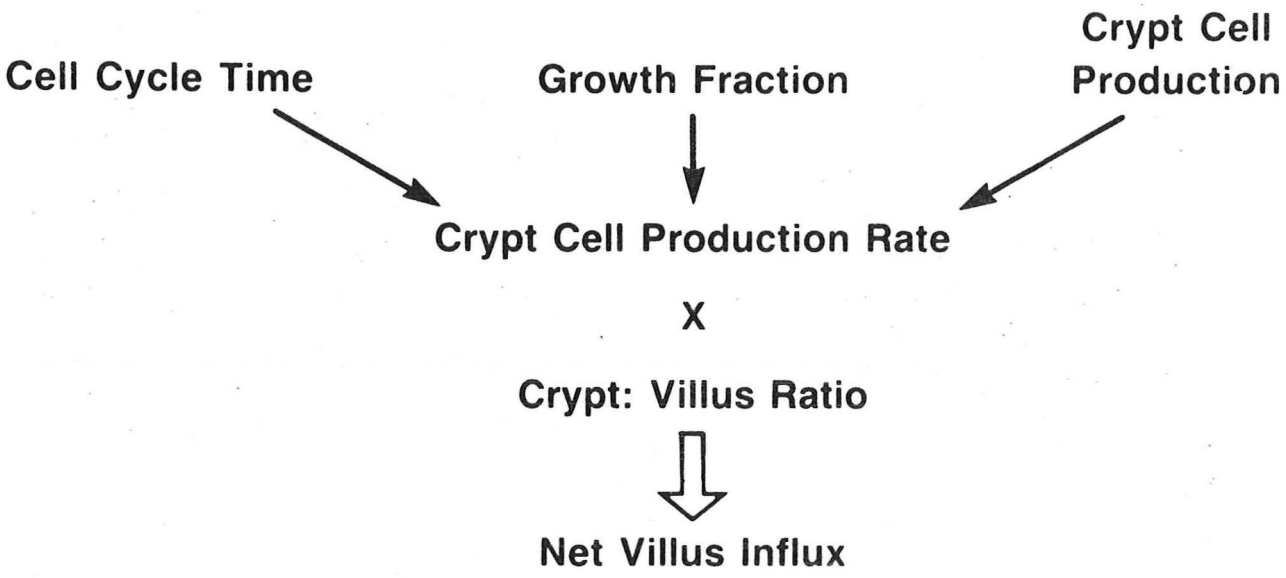

FIGURE 2: *The cell kinetics of the crypts*

The cell cycle time is defined as the interval between two successive divisions of a proliferating cell. The growth fraction is the portion of the cell population devoted to proliferation, and the crypt cell population is the total number of cells in the crypt. The crypt cell production rate may be increased by a reduction of cell cycle time or an increase in the growth fraction or crypt cell population. The net villus influx is determined not only by the crypt cell production rate but also by the crypt:villus ratio, i.e., the number of crypts that open to a villus surface. It is tacitly assumed that in a steady state net villus influx equals cell loss and that mucosal cell population remains relatively constant as illustrated in Figure 3.

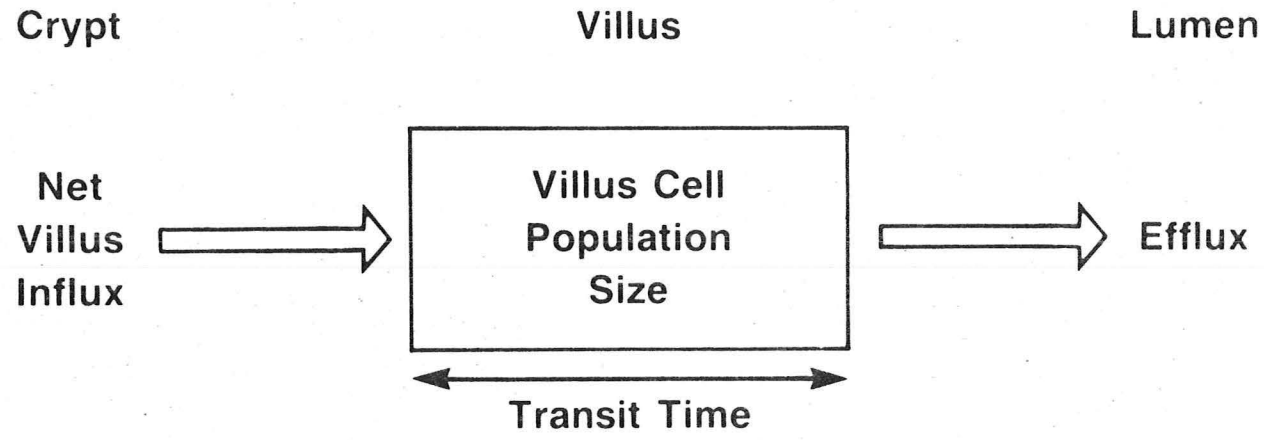

FIGURE 3: *The steady-state relationship between net villus influx and efflux.*

The characterization of the cell kinetics of the intestinal mucosa thus requires accurate determinations of villus cell population size, net villus influx and transit time. This has recently been accomplished by Wright in an elegant study of the intestinal mucosa in mice which will be described in some detail below (24).

The villus cell population size was determined by microdissection of individual villi and crypts. Individual villi or crypts were transferred to a slide in acetic acid and squashed under a cover slip which released the cells from the villus core, and all the columnar epithelial cells were counted. The same procedure was used for the crypts. As shown in Figure 4, there is a linear decrease in cell population size from the proximal to the distal intestine. The method was very reproducible with a small standard error (<5%).

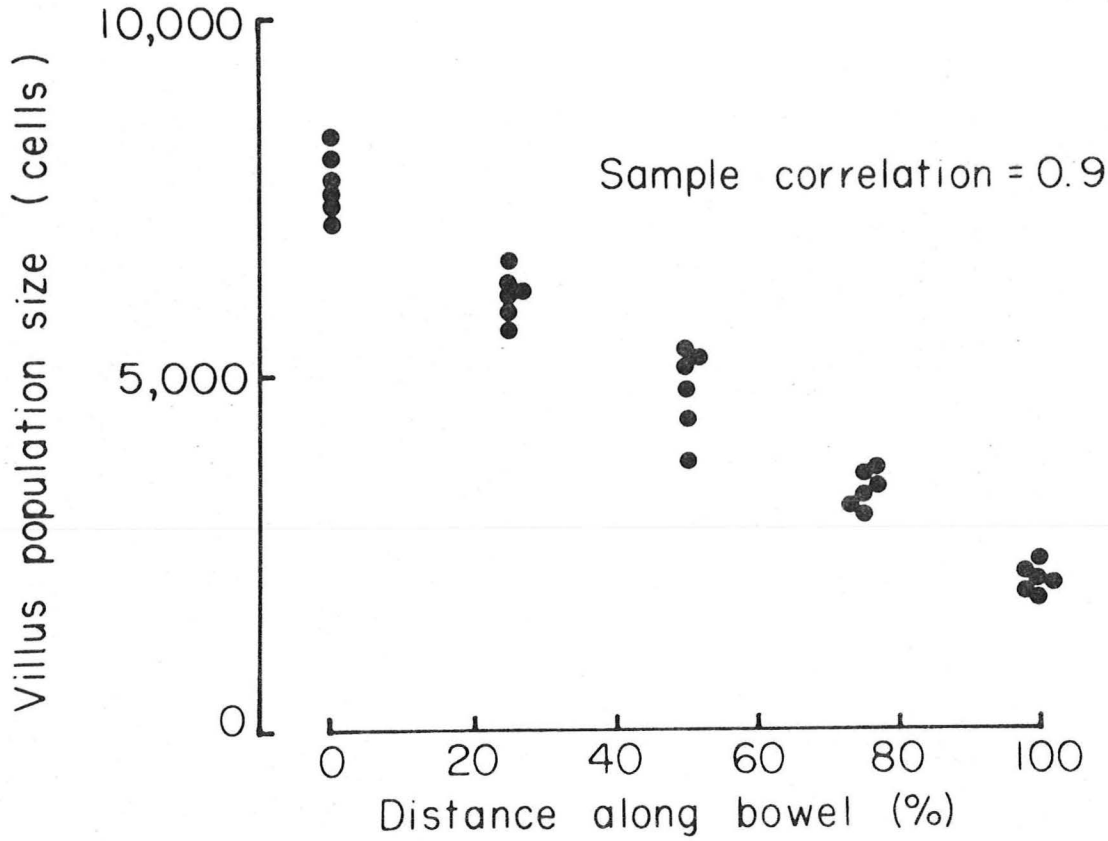

FIGURE 4: *Villus population size in relation to position in the small intestine*

Also, the villus cell population size correlated with more standard morphometric measurements such as villus height and villus row count.

The net villus influx is the product of crypt cell production rate and the crypt:villus ratio. The crypt cell production rate was determined with a stathmokinetic technique by inducing metaphase arrest with vincristine and again using microdissection and counting the number of metaphases in a time-sequence study. The number of metaphases per crypt were plotted against time as shown in Figure 5.

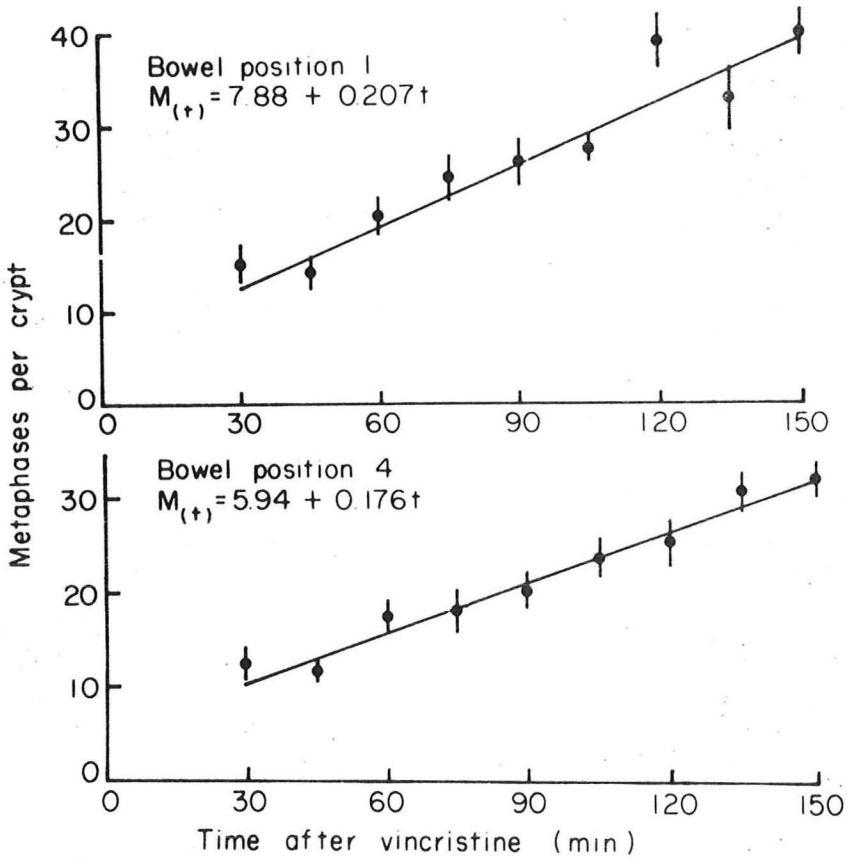

FIGURE 5: *The number of metaphases per crypt for two bowel positions as a function of time after vincristine*

The slope of this line is equal to crypt cell production rate (in cells/crypt per hour). The crypt:villus ratio was determined by counting the number of crypts and villi within a defined area of the mucosa, and these measurements were made in the same block of tissue from which the crypt cell production rate was determined. The crypt:villus ratio also decreased in a linear fashion along the length of the intestine from about 14 in the proximal to about 6 in the distal part (Figure 6).

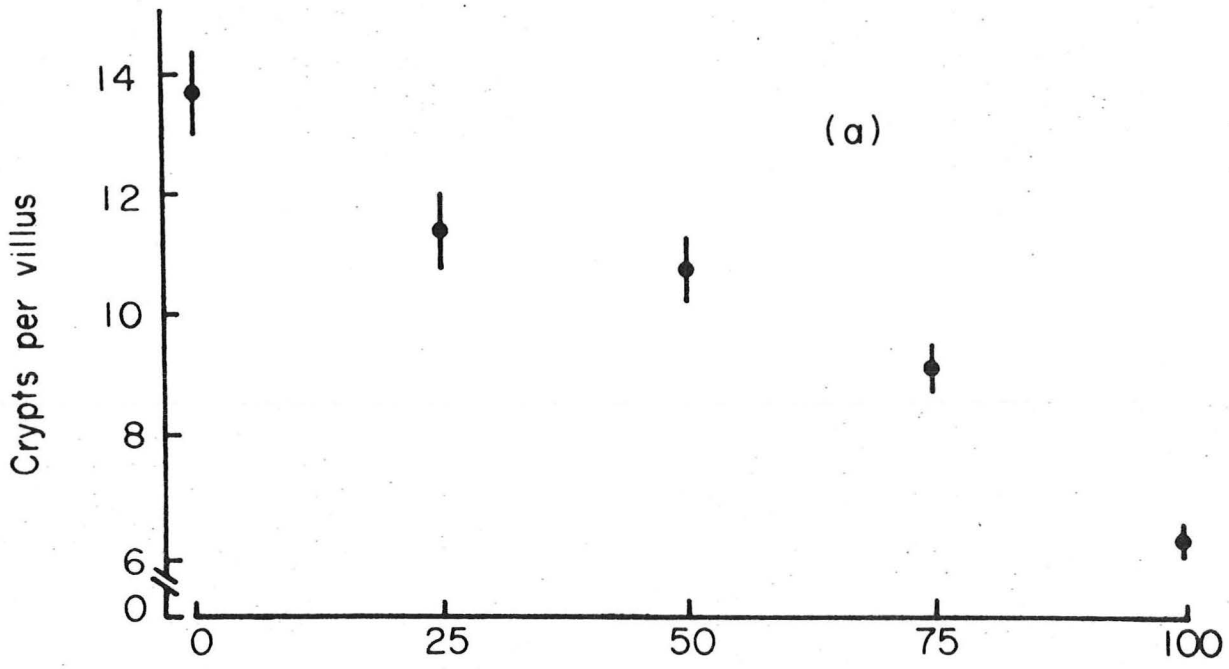

FIGURE 6: *The crypt:villus ratio as a function of position in the small intestine*

From these two measurements the net villus influx could be calculated, which amounted to 170 cells/villus per hour in position 1 and 81 cells/villus per hour at position 5. Over a 24-hour period about 50% of the villus cell mass is renewed at position 1 and almost 90% at position 5.

The villus transit time was determined with ^{3}H-thymidine and autoradiography of cut sections of the villi with counting of the proportion of the labeled cells at the crypt:villus junction and at the villus tips and plotting these numbers against time (Figure 7).

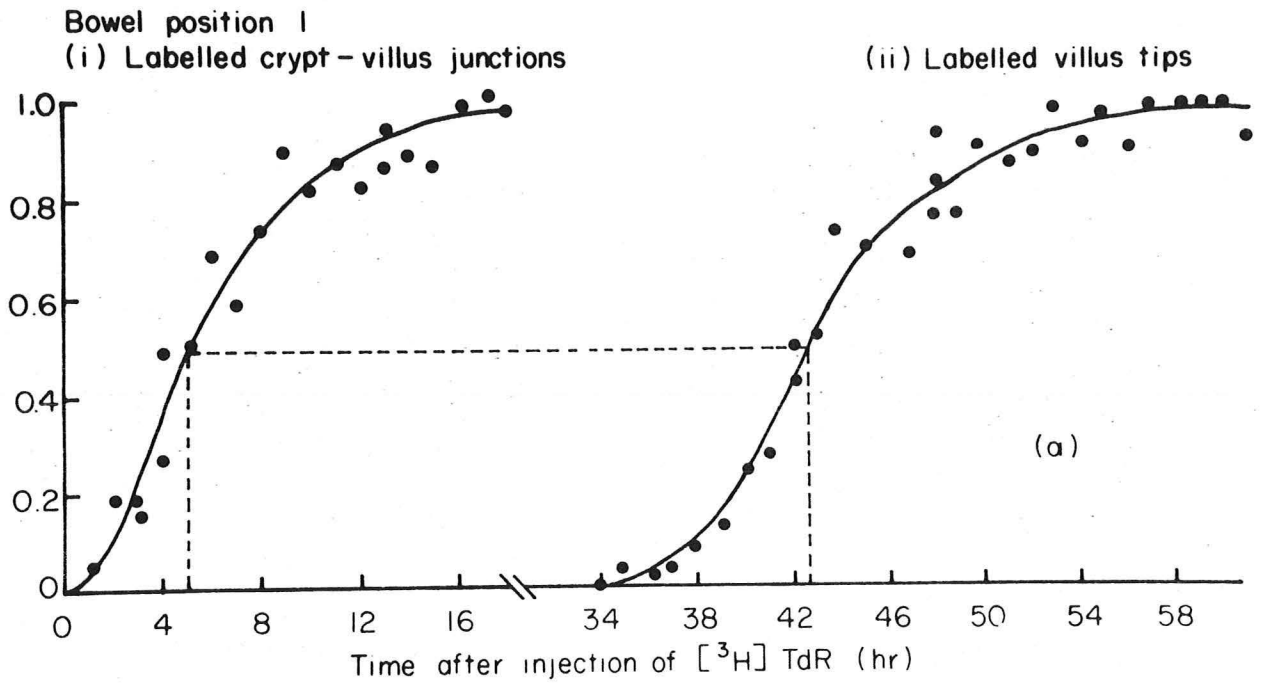

FIGURE 7: *The ratio of labeled cells at the crypt-villus junction and at the villus tips as a function of time after ^{3}H-thymidine injection*

The time for 50% of labeling, indicated by the dashed lines, is the median transit time. The median transit time for the movement of an enterocyte from the crypt:villus junction to the villus tips is found by substraction of these two values. The median transit time in this bowel position (proximal) is about 38 hours.

Finally, the villus cell population size is linearly related to the crypt cell mass (crypt cell population x crypt:villus ratio) with a slope of unity as shown in Figure 8.

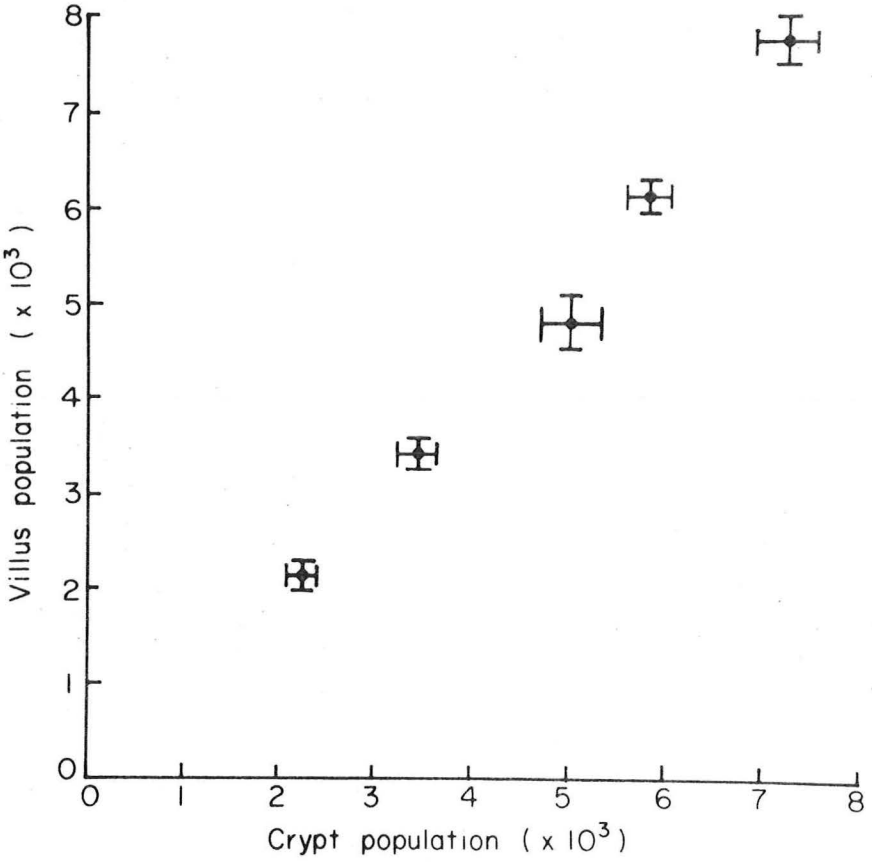

FIGURE 8: *The correlation between crypt cell population and villus cell population*

The equivalency between the crypt cell mass and villus cell population strongly argues for a steady-state condition where, for instance, increased efflux will lead to a compensatory change in net villus influx in an attempt to keep villus population size constant.

Several investigators have claimed that the villus cell population size exerts a negative feed back control on crypt cell proliferation, i.e., a decrease of the villus cell pool will stimulate crypt cell proliferation (25,26). The signals controlling the feed back regulation are completely unknown.

The methodology outlined above allows a precise and accurate description of the important parameters that control the cell kinetics in the mucosa of the small intestine. Wright and coworkers have applied a slightly modified methodology to a study of patients with nontropical sprue; 62 patients with severe changes (flat mucosa) and 47 patients with moderate changes (convoluted mucosa) (27). The results of these studies demonstrate that the net villus

influx is increased by 6.5-fold in nontropical sprue with a flat mucosal lesion as compared to controls, which agrees very favorably with recent studies of cell loss as measured by DNA content of washings from perfused small intestine (6-fold increase in nontropical sprue) (28). In the group of patients with moderate changes the net villus influx was increased almost 3-fold, which is intermediate between normal controls and a flat mucosal lesions.

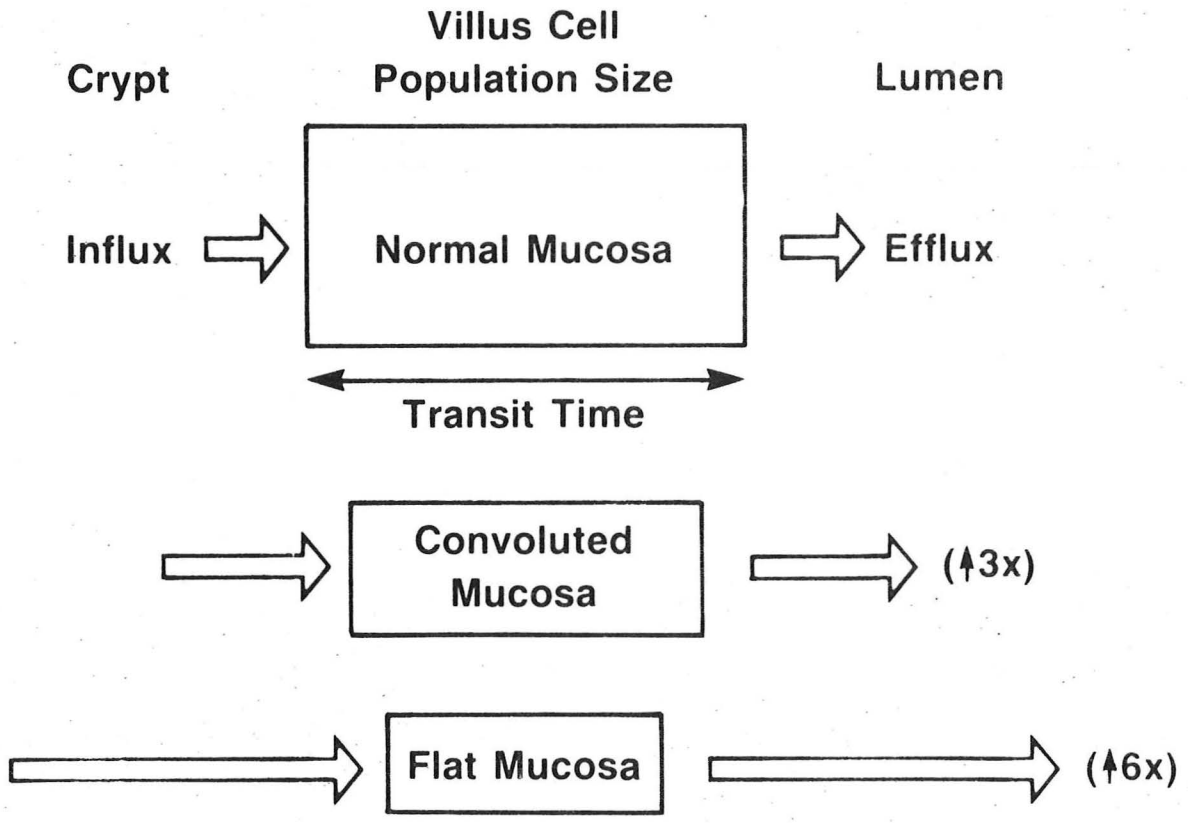

FIGURE 9: *The decrease in villus cell population size as a result of increased rate of efflux*

Accordingly, the following models may be constructed to account for the mucosal changes when the cell kinetics are perturbed by increased efflux (Figure 9).

The model predicts and the experimental data are compatible with the following conclusion. In case of increased efflux a steady state can only be maintained at the expense of a decreased villus cell population size. The intestinal mucosa is therefore converted from a folded to a flat membrane when villus cell death (efflux) is progressively increased.

Similar elegant kinetic studies have not been performed in tropical sprue, but there is every reason to believe that the mucosal changes in this disease (29) are also a result of increased cell death with compensatory crypt hyperplasia according to Booth's original hypothesis (15).

It should be emphasized that a "flat mucosa" is characteristic of the sprue syndromes but not pathognomonic. A variety of gastrointestinal diseases may result in a similar lesion (Table 2) which indicates that the small intestinal mucosa reacts to different toxic injuries with a similar morphological response (30).

CAUSES OF FLAT INTESTINAL MUCOSA

- Nontropical Sprue
- Tropical Sprue
- Acute Enteritis
- Eosinophilic Gastroenteritis
- Cow's Milk Protein Sensitivity
- Immunodeficiency Syndromes
- Giardiasis
- Dermatitis Herpetiformis
- Radiation
- Antineoplastic Drugs

TABLE 2

Etiology and Pathogenesis

A. Nontropical Sprue:

The etiology of nontropical sprue is still unknown. Evidence, however, is accumulating that indicates that both genetic and immunological factors are of importance for providing susceptibility to develop gluten intolerance. First, there is a higher incidence of the disease in first degree relatives of patients with nontropical sprue (31-33). Second, there is a high concordance of the disease in monozygotic twins with a concordance rate of 71% in twin pairs (34). In 1972, Falchuk found that HLA-B8 was highly associated with nontropical sprue with a frequency of 88% as compared to a frequency of 22% in a control population (35). This observation has been confirmed in several studies (36,37) and an association between nontropical sprue and the

haplotypes DR3 or DR7 has also been documented (38-41). Most recently the strongest association has been observed with the DC3 allelic specificity, which is determined by the HLA associated DC locus and was present in 100% in 60 patients with nontropical sprue (42).

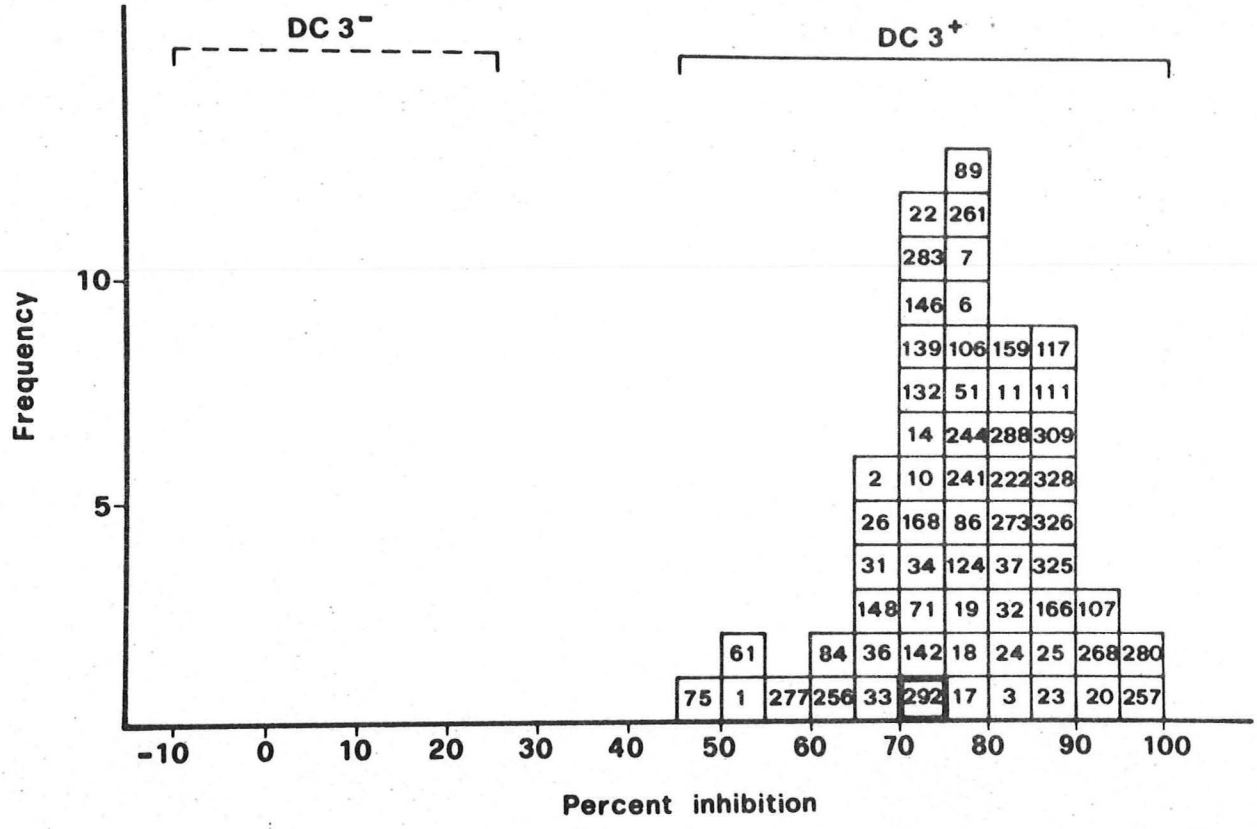

FIGURE 10: *The association between $DC3^+$ and nontropical sprue*

DC3 is also strongly associated with the two DR specificities, DR3 and DR7 and the following haplotypes can be distinguished: 1: $DR3^+$ $DC3^+$; 2: $DR3^+$ $DC3^-$; 3: $DR7^+$ $DC3^+$; and 4: $DR7^+$ $DC3^-$. Only haplotype 1 and 3 ($DC3^+$) was associated with nontropical sprue and the authors conclude that only the $DC3^+$ haplotypes are associated primarily with this disease and that DR3 and DR7 are associated only as a consequence of their linkage disequilibrium with DC3. Thus, the association of nontropical sprue with other HLA specificities follows an order of the degree of linkage disequilibrium as outlined in Figure 11.

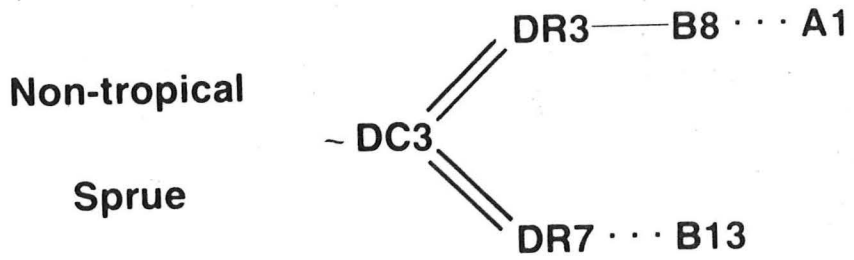

FIGURE 11: *The decreasing order of linkage disequilibrium in nontropical sprue*

The high incidence of the haplotypes HLA B8 and DR3 and DR7 is not unique for nontropical sprue. The same HLA antigens occur at a high frequency in several organ-specific diseases of presumed autoimmune origin (autoimmune chronic active hepatitis, myasthenia gravis, Graves' disease, and insulin-dependent diabetes mellitus). Thus, it has been suggested that the genes that code for HLA-B8 and DR3 are common in these diseases because they determine the host response to environmental factors such as viruses, which has been postulated to initiate this diverse group of diseases (43).

The reason why certain haplotypes confer susceptibility to a specific disease such as nontropical sprue is still poorly understood. It has been claimed that the DC locus controls the induction of T-cell specific killing and that a cellular cytotoxic mechanism may be involved in the destruction of the enterocytes, but this still awaits definite proof (44).

However, the HLA associated specificities are not the only genetic factors involved in the development of nontropical sprue, because only <0.2% of the individuals with these specificities develop the disease. Other HLA-associated diseases are best explained by a dual genetic control (45,46). If a second locus is involved, two possible candidates may be either a non-HLA linked B-cell specific antigen (45) or the immunoglobulin heavy chain allotype marker G2m, which is strongly associated with the presence of IgG antibodies against gliadin (43). Alternatively, it has been claimed that genetic factors alone are not the sole requirement for disease development since there is not complete concordance in identical twins and that environmental factors (virus

infection) are required to precipitate the disease, such that ingestion of gluten becomes toxic for the small intestinal mucosa.

One of the histologic characteristics of sprue is the increased number of lymphocytes and plasma cells in the lamina propria and in the epithelium. It has recently been recognized that the increase in inflammatory cells is more apparent than real. With the use of more sophisticated morphometric techniques, it was found that there is no quantitative difference in the number of inflammatory cells between patients with nontropical sprue and normal controls (47-51). Two recent studies have looked at qualitative differences by quantifying the proportions of lymphocyte subpopulations (49, 52) intraepithelially. 80% of the intraepithelial lymphocytes are T-cells of which 80-90% are of the suppressor type and only 10-20% of the helper-inducer type. A statistical significant difference between the subpopulations in controls and nontropical sprue could not be demonstrated. The functional capacity of the intraepithelial lymphocytes has been addressed in a few studies. In one study it was found that the lymphocytes in intestinal mucosa from untreated nontropical sprue, maintained in organ culture with gluten, produced lymphokines which inhibited leucocyte migration. Intestinal mucosa from controls and from patients in remission on gluten-free diet did not elicit migration inhibition (53). The results of these studies might indicate that gluten induces a T-cell sensitization in untreated nontropical sprue. In another study it was found that HLA DR3 and DR7 confer a T cell hyporesponsiveness to gluten (54). These conflicting results serve to emphasize that the immunological reactions involved in nontropical sprue are still poorly understood.

It has been well documented for 35 years that removal of gluten from the diet in patients with nontropical sprue will result in clinical and morphological improvement. It is less clear, however, why a single protein suddenly becomes toxic whereas thousands of other proteins can still be ingested without harm. One might ask why gluten, like other proteins, are not broken down by pancreatic proteolysis to individual amino acids and dipeptides which are then absorbed? An early theory, promulgated by Frazer (55,56) was that patients with nontropical sprue lacked a peptidase required for gluten breakdown ("the missing peptidase hypothesis"). Many studies have been performed to confirm or reject this hypothesis. The proteins in gluten are relatively resistant to proteolysis by pepsin and trypsin and even exhaustive in vitro digestion with these enzymes is incomplete leaving several large peptide fragments (57). The final steps in the breakdown of these fragments are accomplished by peptidases in the brush-border (58). Recently it has been confirmed that asymptomatic patients on a gluten-free diet have normal peptidase activities in the microvillus membrane and that the ability to completely digest gluten peptides was identical to controls (59). Thus, the "missing peptidase" hypothesis has finally been put to rest, but the basic problem of why gluten is toxic in susceptible individuals still remains to be solved.

Gluten is found in wheat, rye, barley, and possibly oats (60). The two main constituents of gluten are gliadin and glutenin, and it is the gliadin fraction which contains the toxic protein or peptide. The gliadin fraction is usually defined as that portion of gluten which is soluble in 70% ethanol. Gliadin may be subdivided in four main fractions on starch gel

electrophoresis: the α, β, γ, and ω fractions. Many studies have been performed with clinical testing of these four fractions in order to define the toxic peptide and the results have been conflicting. Some investigators found all four fractions were toxic (61,62) whereas others found the toxicity confined to the α-fraction (63). These conflicting results were presumably due to inadequate chemical separation and with improved separation it has recently been shown that only α-gliadins are toxic to the intestinal mucosa (64). Kasarda and his group have recently succeeded in determining the primary sequences of α-type gliadins by DNA sequencing of cDNA clones and compared that to the amino acid sequence of A-gliadin, which is an aggregable type of α-gliadin (65). The main structure of the protein can be divided into 5 domains as shown in Figure 12.

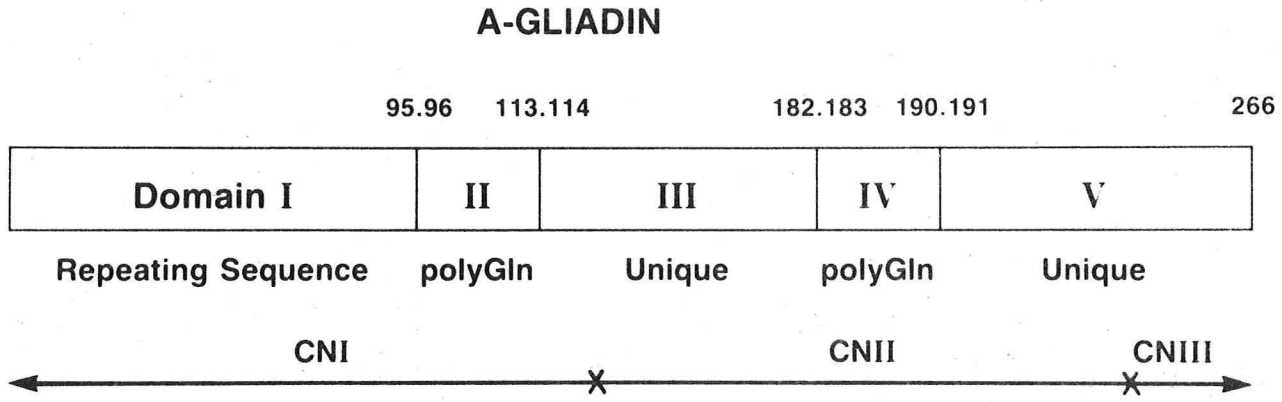

FIGURE 12: *The five domains of A-gliadin*

The protein is rich in glutamine and proline and contains 266 amino acids. Domain III and V contain unique sequences, whereas domain II and IV are esssentially polyglutamines. Kasarda has also prepared three cyanogen bromide peptides that span the entire A-gliadin molecule (CNI 1-127; CN II 128-246 and CN III 247-266). With this information in hand they took a novel approach and looked for structure homology between the A-gliadin peptide and other proteins and screened A-gliadin against about 1500 proteins (minimal requirement: at least 8 or more amino acid identities within a 20 residue segment) (66). Eleven proteins were detected of which 10 were considered not to be significant. The only protein that shared significant sequence homology with A-gliadin was the early region E1b protein of human adenovirus type 12 (Ad 12) as illustrated in Figure 13.

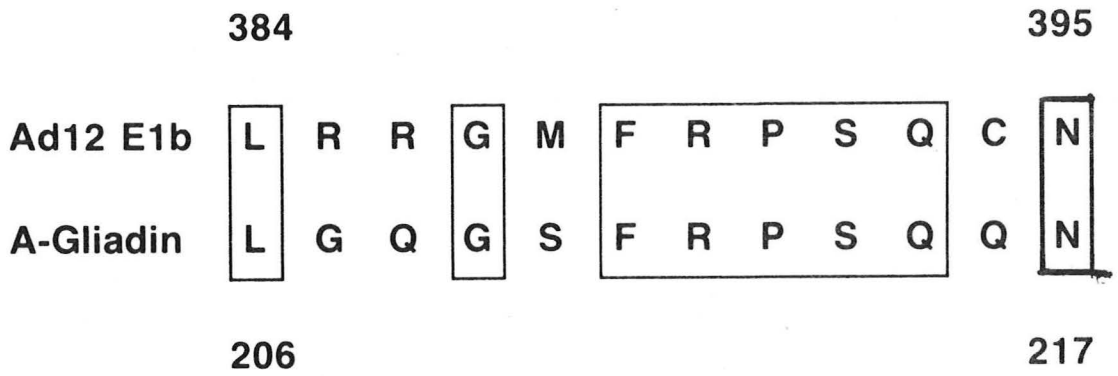

FIGURE 13: *Amino acid sequence homology between the E1b protein and A-gliadin*

Within the 12 amino acid sequence there were 8 amino acid identities with an uninterrupted 5 residue sequence. The sequence homology occurred within domain V of A-gliadin (Figure 12). With a variety of techniques they could further demonstrate that antibodies raised against the Ad12 E1b protein cross-reacted with A-gliadin, the CNII peptide and a synthetic heptapeptide with sequence 211-217 of A-gliadin. Conversely, a monoclonal antibody against the synthetic heptapeptide reacted with Ad12 E1b protein.

Adenovirus type 12 is a human virus which is isolated from the intestinal tract and is usually not implicated in human disease. Recently, it has been shown that cells transformed by Ad12 induce tumors in rodents at a high frequency (67) and that cells infected by Ad 12 have a decreased expression of Class I major histocompatibility complex antigens on the cell surface, which would render these cells less susceptible to T cell killing (68).

The peptide homology between Ad12 E1b and A-gliadin is probably due to chance because these proteins are not related functionally and do not share a common ancestry. However, based on the above findings, Kasarda proposed an intriguing theory on the etiology and pathogenesis of nontropical sprue as

follows: the association with specific HLA haplotypes may be of importance for the host's immune response to specific viral infections. The encounter of the immune system with a specific protein produced during viral infection may play a role in the pathogenesis of nontropical sprue because of the chance immunological cross-reactivity between the viral protein and gliadin. Thus, ingestion of gliadin may then serve as a target for immune-mediated injury of the intestinal mucosa.

It is apparent that many questions regarding the etiology and pathogenesis of nontropical sprue remain to be answered, but also that some progress has been made.

B. Tropical Sprue

The evidence accumulated so far suggests that tropical sprue is an infectious disease with persistent contamination of the small bowel by enteric pathogens (69). The evidence is based on the following findings:

1. The disease usually follows an episode of acute diarrhea (turista) at least in visitors to the endemic areas. The development in native residents is not well documented.

2. Seasonal epidemics of acute diarrhea of unknown etiology have been followed by the development of tropical sprue (70,71).

3. Household epidemics of acute diarrhea followed by the development of sprue have been observed (72).

4. In most patients studied an overgrowth of coliform bacteria (Klebsiella, E. coli and Enterobacter) in the proximal small intestine has been found (73,74).

5. Tropical sprue responds to treatment with antibiotics (tetracycline).

It might be argued that the presence of bacteria in the small bowel only reflects a constant exposure to a contaminated environment, but most healthy native residents do not have bacteria in the proximal small intestine (75). Furthermore, the bacterial contamination is not a mixed population but is most often a single or a few specific species or serotypes (76). These coliform bacteria are noninvasive and do not produce heat-labile or heat-stable enterotoxins by conventional assays similar to those produced by enterotoxigenic E. coli or Klebsiella (77). When strains of these coliform bacteria, isolated from patients with tropical sprue, are instilled into a ligated rabbit loop or perfused in vivo through the rat small bowel they result in fluid secretion and with time in morphological changes with broadening and shortening of the villi (78). The enterotoxin(s) that cause these effects have so far only been partly characterized (79).

Tropical sprue may then represent the sequella of acute diarrhea due enterotoxigenic coliform bacteria which for unknown reason in some persons persist and with time produce mucosal lesions and malabsorption. The reason why the bacteria persist in some, whereas in most persons turista is a

self-limited disease, is unknown. It has been observed that the bacterial counts are higher in the mucosa than in the luminal fluid which may relate to their capacity to adhere to and proliferate on the mucosal surface (80), and the question has been raised whether patients who develop tropical sprue have an inadequate local immune response? It has been well documented that the severity of the mucosal lesion is related to the bacterial counts, i.e., the villus efflux due to cell death is directly related to the burden of bacterial contamination (74,80). An animal model of tropical sprue has not been established. The oral administration of these coliform strains to suckling piglets causes acute diarrhea, but the piglets were not observed for an extended period of time to determine whether they would develop a disease resembling tropical sprue (81).

As in nontropical sprue, there are several questions that remain unexplained in tropical sprue, but the pathogenesis of the mucosal lesion appears to be a result of a prolonged contamination of the small intestine with coliform bacteria.

The Pathophysiology of the Malabsorption Syndrome

Both tropical and nontropical sprue are characterized by panmalabsorption. The cause of the malabsorption was previously claimed to be the result of loss of absorptive surface area when the mucosa is converted to an essentially flat surface. It has been estimated that the presence of villi increase the surface area by 7-14-fold. If absorption of nutrients took place on the whole surface of the villus, it would appear reasonable that a 7-14 fold reduction of surface area would cause malabsorption of a variety of substances. This explanation is probably not entirely correct. All experimental evidence suggests that mainly the tip area of the villi is engaged in the absorptive process and that the stem of the villus merely serves as a maturation zone for the enterocytes for the development of full functional capacity. The evidence is based on both functional studies of intestinal transport and biochemical studies of enzyme activities at different locations on the villus (82-84). In other words the absorptive surface area can be viewed as a cylinder touching the tips of the villi as shown in Figure 14. The area of the cylinder in the sprue syndromes must be slightly larger due to an increase in diameter.

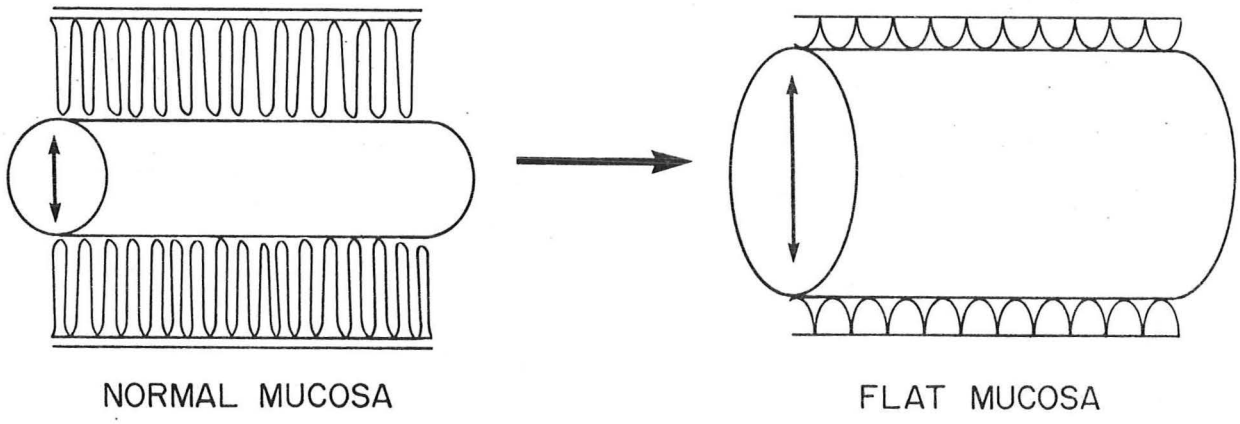

FIGURE 14: *The cylindrical absorptive surface area in normal intestinal mucosa and in sprue*

The reason for the profound disturbances in the absorptive processes is found at the cellular level. In the sprue syndromes the normal mature enterocytes have been replaced by a population of immature crypt cells. On light microscopy these cells are small cuboidal cells as opposed to the tall columnar cells normally present on the villus. The cells appear irregular in size and shape when examined by EM or SEM. Moreover, the microvilli are disorganized and irregular, and the glycocalyx is poorly developed.

The microvilli serve as a structural support for a number of enzymes (peptidases, disaccharidases) involved in the final steps of protein and carbohydrate digestion; multiple specific transport carriers (glucose, amino acids, sodium, chloride, etc.) engaged in the translocation of these molecules across the microvillus membrane and into the enterocytes. The microvilli on the immature crypt cells are not as abundant as on the mature cells, and disaccharidase and dipeptidase activities are markedly decreased (85,86). The kinetics of glucose or amino acid transport have not been well characterized in sprue. Infection of piglets with transmissible gastroenteritis virus produces an acute lesion very similar to sprue (i.e., flat mucosa) with peaks at 40 hours after which recovery takes place (87). Studies of glucose transport into microvillus membrane vesicles at 40 hours demonstrate that

carrier-mediated glucose transport is absent but reappears with recovery. It is therefore entirely feasible that the number of transport carriers are reduced, if not absent, in patients with a flat mucosa, which would further contribute to the malabsorption of amino acids, hexoses and electrolytes. Thus, the immature cuboidal cells with disorganized microvilli covering the flat surface have a severely reduced functional capacity, both qualitatively and quantitatively, to subserve peptide and disaccharide digestion and absorption. A clinical corollary of these findings is the frequent observation of lactose malabsorption in sprue.

Fat malabsorption is a prominent feature of the sprue syndromes, and the derangement of the multiple steps of normal fat absorption, illustrated in Figure 15, is complex.

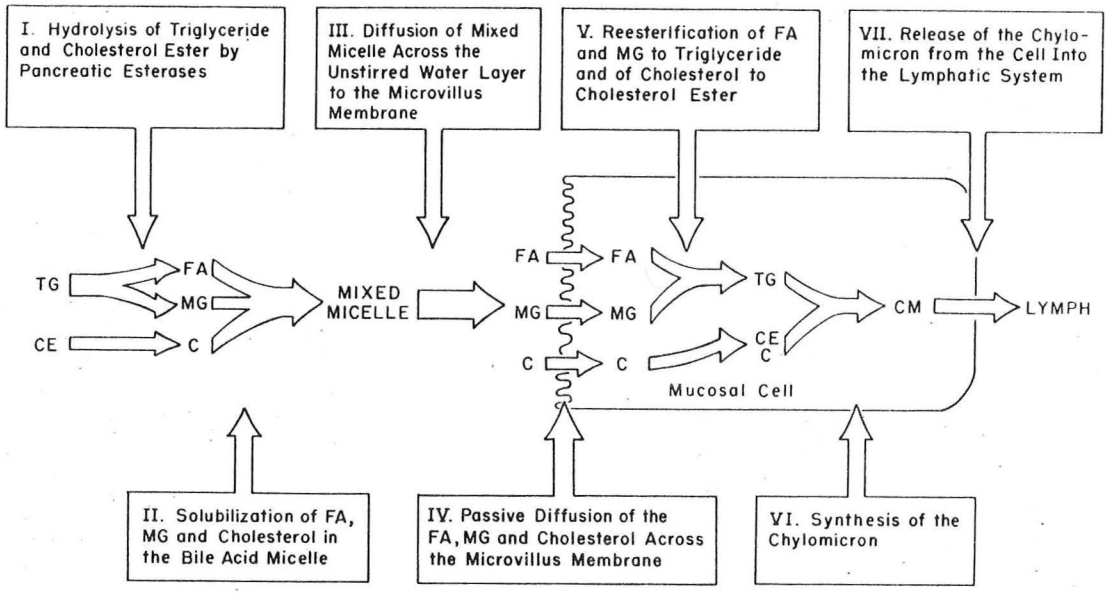

FIGURE 15: *The sequential steps in normal intestinal fat absorption*

In a study of the intraluminal phases of fat digestion in nontropical sprue both pancreatic and gallbladder functions were impaired with decreased lipase output and decreased bile acid concentrations, presumably as a result of inadequate endogenous cholecystokinin stimulation because exogenous CCK would correct the impairment (88). Furthermore, the bile acid concentrations fell

progressively with time in the sprue patients due to excessive intestinal secretion of water and electrolytes. Thus, both impaired lipolysis and decreased micellar solubilization may contribute to fat malabsorption.

At present, the information about the further steps in fat absorption is limited. The passive permeability properties of the microvillus membrane in these immature cells have not been studied. Furthermore, the functional capacity of the enzymatic machinery for triglyceride and chylomicron synthesis has not been investigated. It is entirely feasible that these steps may also be compromised in sprue.

Several investigators have claimed that the intestinal mucosa is "leaky" in nontropical sprue (89-92). The leakiness, however, is peculiar because larger molecules such as lactulose, cellobiose or EDTA (MW:300-350) are more permeable whereas smaller molecules like D-xylose or mannitol and (MW:150-180) are less permeable. These molecules presumably permeate through "pores" in the membrane and through tight junctions. EM studies of the tight junctions indicate that these structures are poorly developed in nontropical sprue (93) which could account for the leakiness. If this is the case, then the tight junctions should be equally permeable to small and larger molecules. The differential permeability changes observed in these studies are poorly understood.

Fordtran has performed intestinal perfusion studies in patients with sprue, and the following observations were made (94): First, the intestinal mucosa in sprue is in a net secretory state as opposed to controls who are in a net absorptive state. Furthermore, addition of glucose to the perfusate did not affect the secretory state. In normal controls glucose stimulates sodium and water absorption by the coupled transport mechanism. This is further proof that the glucose carrier is functionally absent on these immature cells. Second, the effective size of the water-filled pores in the membrane were decreased which would explain the decreased permeability of D-xylose and other smaller molecules in sprue.

In summary, the pathophysiology of the absorptive processes in sprue is multifactorial. The intraluminal digestive process is deranged due to inadequate pancreatic enzyme secretion and the rate of enzymatic breakdown of proteins, carbohydrates and lipids is decreased. Furthermore, the micellar solubilization of the lipolytic products is compromised due to sluggish gallbladder emptying. At the cellular level, there is a marked decrease in enzymatic activities (dipeptidases and disaccharidases) and presumably a similar decrease in the number of transport carriers. Finally, the immature cells are in a net secretory state, and the functional capacity of the intracellular enzymes engaged in triglyceride and chylomicron synthesis may not be fully developed. Thus, almost every step in the normal absorptive process is compromised in the sprue syndromes and results in panmalabsorption.

The Clinical Presentation

A. Tropical Sprue

The diagnosis of tropical sprue should be suspected in a patient who has visited or lived in the tropics and has developed chronic diarrhea (>2 months) with or without clinical evidence to suggest malabsorption. Before embarking on a major diagnostic workup for malabsorption, a parasitic infection (giardia, strongyloides, coccidia) should be excluded. The malabsorption syndrome can be documented with a limited number of tests which include 72 hours fecal fat collection, D-xylose tolerance test and a Schilling test (I and II). If malabsorption is confirmed by increased fecal fat and decreased D-xylose and vitamin B_{12} absorption, then a small bowel biopsy should be performed to document morphological changes compatible with sprue. The morphological changes, however, are not diagnostic of tropical sprue, and the conclusive proof of the diagnosis is the response to treatment with folic acid (5 mg/day) and tetracycline (250 mg q.i.d.) which will result in clinical improvement within two weeks. It is usually recommended that the medical treatment should be given for several months, and complete recovery should be expected.

The incidence and prevalence of tropical sprue among the indigenous population in the tropical countries is unknown, but it has been assumed that millions of people have the disease in a chronic form and that tropical sprue contributes significantly to increased morbidity and malnutrition in these countries.

B. Nontropical Sprue

The clinical recognition of nontropical sprue in an adult was previously based on the demonstration of frank malabsorption in a person who had not visited the tropics and in whom other causes of malabsorption had been excluded using the same set of diagnostic tests as in tropical sprue and then proceeding to small bowel biopsy. Again, the flat mucosal lesion is compatible with but not diagnostic of nontropical sprue. The proof of the diagnosis is the response to a gluten-free diet. In the majority of patients clinical improvement will occur within weeks, but morphological improvement may take months and complete morphological recovery is unusual, which may indicate that most patients do not adhere strictly to the diet or that it is impossible to avoid ingestion of small amounts of gluten. It is generally recommended that the patients continue with a gluten-free diet throughout life.

The pattern of presentation of nontropical sprue appears to have changed over the last 10-15 years mainly due to increased recognition. In countries where the incidence of the disease is high, many patients now present without specific gastrointestinal symptoms. In a series of 102 patients with nontropical sprue, seen in Edinburgh from 1975-79, 56 patients had no gastrointestinal complaints and only 13 presented with a typical malabsorption syndrome (95). The suspicion of nontropical sprue in the patients without gastrointestinal symptoms was raised because of hematologic abnormalities (macrocytosis or iron-deficiency anemia without evidence of blood loss),

evidence of bone disease (osteoporosis/osteomalacia with hypocalcemia or elevated alkaline phosphatase), short stature or due to conditions known to be associated with sprue (oral aphthae or jejunal ulcerations). Similar conclusions were reached in a series of 88 patients from England (96) where only 26% had classic symptoms of malabsorption. It may be that nontropical sprue has a long, fluctuating course with only subclinical symptoms. At any rate, increased awareness of the disease and the availability of small bowel biopsy has lead to an earlier recognition of the disease.

A minority of patients will either not respond to gluten-free diet or may respond initially but at a later date deteriorate despite adherence to the diet. The disease in these cases is sometimes called refractory sprue. Some of these patients will respond to treatment with corticosteroids in addition to the diet and will require steroids for a prolonged time. Another even smaller group of patients will not respond to gluten-free diet or steroids, and the disease is often fatal. In these patients it may be wise to revise the diagnosis and search for other lesions such as intestinal lymphoma. Alternatively, the mucosal lesion may not be due to gluten but to other substances ingested in the diet. An interesting case report was recently published on a patient who had malabsorption and a flat jejunal mucosa (97). She did not respond to a gluten-free diet or steroids. She was then placed on parenteral hyperalimentation and was tested with one nutrient at a time and was found to react to eggs, chicken and tuna fish. Exclusion of these three nutrients resulted in clinical improvement and morphological remission of the jejunal mucosa. She refused a rechallenge with gluten. Thus, it is not known whether she had nontropical sprue with secondary reactions to these three nutrients or whether the flat mucosal lesion was unrelated to gluten and produced by an unidentified peptide. It seems likely that the mucosal lesion seen in some of the patients with refractory sprue is not a reaction to gluten but to still unidentified peptides.

Finally, within the groups of patients with refractory sprue, another entity was recently defined, socalled "collagenous sprue" characterized by a dense band of collagen at the basal membrane of the epithelial cells (98). It has been questioned, however, whether these patients represent a separate entity. In a large series of patients with nontropical sprue an increased amount of collagen was present in 30-40 percent of cases in initial biopsies (99). In patients who responded to gluten-free diet the collagen disappeared, whereas it persisted in refractory cases. Thus, the persistence of collagen may merely indicate that the disease is refractory to treatment and has a very poor prognosis.

Diseases Associated with Nontropical Sprue

A. Dermatitis Herpetiformis

The association of dermatitis herpetiformis and nontropical sprue was first documented in 1966 (100) and has been verified by several studies of larger series of patients since that time (101,102). Dermatitis herpetiformis is a blistering skin disease characterized by granular deposits of IgA in the dermal papillae of uninvolved skin. The skin lesions usually respond to treatment with dapsone. The incidence of HLA haplotypes B8 and DR 3 and 7 occurs with the same high frequency in dermatitis herpetiformis as in

nontropical sprue. There has been some disagreement about the frequency with which mucosal changes similar to sprue occurs in dermatitis herpetiformis (103). In recent large series, however, the issue has been settled and an incidence of 70-80% was documented. The lesions are often patchy and require multiple biopsies for documentation. Malabsorption with diarrhea and weight loss is exceedingly rare in dermatitis herpetiformis and only a minority will have abnormal absorption tests (104). This probably reflects the patchiness of the mucosal lesion which also may have a limited extension in the small bowel. The mucosal lesion responds to a gluten-free diet. Furthermore, gluten-free diet also appears to have a beneficial effect on the skin lesions in that about 50% will be able to stop the dapsone medication completely, another 30% will be able to reduce the dapsone requirement and only about 20% remain unchanged (101). The response of the skin lesions to the diet is slow and may take 1 to 2 years, whereas the mucosal changes improve within six months (105). The exact relationship between nontropical sprue and dermatitis herpetiformis is obscure. IgA circulating immune complexes are significantly increased in patients ingesting gluten. It has been speculated that gluten stimulates IgA synthesis in the intestine, which then result in a rise in IgA immune complexes by release to the blood and for unknown reasons precipitation in the dermal papillae (104). The association between these precipitates and the skin lesions is also not clear as the IgA precipitates persist long after the skin lesions have cleared on a gluten-free diet.

B. Malignant diseases

The observation that nontropical sprue may be associated with malignant diseases, both intestinal and extraintestinal, and that the incidence of malignancy may be higher than in a background population was initially documented by case reports and small series of patients. In 1978, a collaborative study was started in England to collect all documented cases of nontropical sprue associated with malignancies throughout the United Kingdom. About 400 cases were collected, of which 235 fulfilled the acceptance criteria (106). In the 235 patients, 259 histologically confirmed malignancies were found (215 had single tumors whereas 20 had two or more). 133 of the tumors were malignant lymphomas which were classified as listed in Table 3.

MORPHOLOGICAL CLASSIFICATION OF MALIGNANT LYMPHOMAS

	No
Well-differentiated Lymphocytic	2
Undifferentiated Large Cell	9
Histiocytic	107
Mycosis Fungoides	1
Unclassified	14
Total	133

TABLE 3

Histiocytic lymphoma was by far the most frequent tumor (90%). The diagnosis was established in life in 84 patients with malignant histiocytosis, and the presenting localization is shown in Table 4.

PRESENTING SITE OF MALIGNANT HISTIOCYTOSIS DIAGNOSED IN LIFE

	No
Superficial Lymphnodes	5
Thorax	3
Stomach	1
Small Intestine	67
Colon	2
Other Intra-abdominal Sites	3
Other	3
Total	84

TABLE 4

The tumor was localized to the small intestine in about 80% of these patients.

Among the remaining 123 histologically classifiable tumors which were nonlymphomatous, 60 tumors were localized to the gastrointestinal tract with a distribution as shown in Table 5.

OBSERVED AND EXPECTED NUMBERS OF GASTROINTESTINAL MALIGNANCIES

	Observed	Expected
Mouth	3	1.55
Pharynx	4	0.69 *
Esophagus	10	2.04 *
Stomach	7	7.01
Small intestine	19	0.23 *
Colon	6	7.77
Rectum	7	5.38
Other	4	4.51
Total	60	

TABLE 5

In this table is also listed the observed and expected frequences of malignancy at the respective sites. Of particular interest is the finding that the incidence of adenocarcinoma of the small intestine, which is very rare, was significantly increased with a relative risk of 82.6. The true incidence of malignant disease in nontropical sprue is not known since the total number of patients with nontropical sprue living in the United Kingdom at the time of the study cannot be assessed. Furthermore, the important question, whether a clinical response and strict adherence to a gluten-free diet protects against development of malignancies, could not be answered by the study because the number of patients who had never received a gluten-free diet was too small for comparison and statistical analysis. In anecdotal reports, however, it has been observed that intestinal lymphoma can develop in patients who responded clinically and morphologically to a gluten-free diet (107). The increased risk of malignancies in nontropical sprue should serve to increase the suspicion of a potential malignant disease in patients who deteriorate despite adherence to the diet.

C. Intestinal ulcers and strictures

The rare disease "chronic nongranulomatous ulcerative jejunitis" also has a peculiar association with nontropical sprue. This disease is characterized by chronic, multiple, apparently benign ulcers most frequently localized to the jejunum. The ulcers may be complicated by hemorrhage, perforation or stricture formation, which often are the presenting features of the disease and the diagnosis is established at laparotomy. The majority of patients with this rare disease have a flat jejunal mucosa identical to nontropical sprue. When patients present with this complication many do not respond to gluten-free diet and are classified as refractory sprue. Intestinal ulceration may also occur in patients with nontropical sprue who responded to gluten withdrawal and then developed this complication while in apparent remission. The prognosis for these patients is generally poor. In a series of 33 patients with intestinal ulcers and malabsorption, 24 patients died of complications (108). Some patients may benefit from intestinal resection when only a limited segment of the jejunum is involved.

In summary, tropical and nontropical sprue both present clinically with malabsorption and morphologically with a flat intestinal mucosa. The two diseases differ, however, with respect to etiology, pathogenesis and treatment. Tropical sprue is an infectious disease and the intestinal lesion is caused by a still poorly characterized enterotoxin. Nontropical sprue on the other hand has distinct genetic and immunological features that presumably renders some persons susceptible to a peptide within the α-gliadin molecule. The molecular events that lead to increased enterocyte turnover still remain to be accurately defined. Tropical sprue is easily cured by antibiotic treatment whereas most patients with nontropical sprue must stay on a gluten-free diet for life.

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