TROPICAL SPRUE is a primary malabsorption syndrome affecting residents of, or visitors to, certain tropical areas. The jejunal mucosa of such patients shows alterations in the shape and orientation of enterocytes, crypt hypertrophy, a reduction in villous height, and marked mononuclear infiltration of lamina propria and epithelium. At the ultrastructural level, intracellular changes within enterocytes of surface and crypt epithelium are apparent, and such cells are often surrounded by collections of lymphocytes; thus it seems clear that damage to the enterocyte is the basic lesion responsible for the malabsorption.

Biopsies of proximal jejunum in over 1500 South Indian patients with tropical sprue have not revealed any with the "flat" mucosa characteristic of untreated celiac sprue. In a recent immunologic study of tropical sprue, counts of epithelial lymphocytes, expressed per 100 crypt and surface epithelial cells, were found to be raised. Such findings are similar to those previously reported both in celiac sprue and milk hypersensitivity, conditions where immunologic mechanisms of pathogenesis are thought to be operative. On the other hand, counts of epithelial lymphocytes in celiac sprue mucosa, when expressed in relation to a fixed area of muscularis mucosae (ie, "areal density") were found to be significantly reduced, compared with appropriate controls. Despite this apparent contradiction, when additional morphometric techniques developed to investigate lymphocyte morphology and kinetics within epithelia were applied to the study of celiac sprue mucosa, clear evi-
lymphocyte "activation," compatible with a local cell-mediated immune reaction, was obtained. It was therefore felt that the possible immunologic component of the tropical sprue lesion, indicated by raised epithelial lymphocyte counts, might be better understood if a similar detailed morphologic analysis of the epithelial lymphocyte population in this condition were undertaken. The results of such a study, presented in this paper, confirm that marked changes are found among the surface and crypt epithelial lymphocyte populations of the upper small intestinal mucosa in tropical sprue, and show that these changes are secondary to the primary damage to the enterocytes.

Materials and Methods

Patients Studied

Thirty jejunal mucosal biopsies from four groups of subjects were studied.

Control Group

Eight subjects, all with normal intestinal function, were included in this group. Four were patients with gastrointestinal complaints but without malabsorption, and the remainder were volunteers from the same village from which the subjects with epidemic tropical sprue (Group II) were obtained.

Epidemic Tropical Sprue

This group comprised 7 patients from a village 25 miles south of Vellore, where an epidemic of tropical sprue occurred between 1975 and 1981; all were symptomatic for less than 1 month (range 4–28 days).

Endemic Tropical Sprue

Patients with endemic tropical sprue present spo-

radically with a primary malabsorption syndrome without any relationship, either in time or place, to other such patients. From among many endemic sprue patients diagnosed in the Unit, 11 patients were selected who were symptomatic for periods ranging from 6 months to 9 years and whose ages approximately matched those of other groups; in this way a representative group of endemic sprue patients was obtained.

Subjects Who Had Recovered From Sprue

Four subjects, 3 of whom were included in Group III, all of whom were now asymptomatic and without demonstrable malabsorption, were selected; histologically their jejunal mucosa was virtually normal.

Control subjects and patients with active disease were admitted to a metabolic ward and investigated as described elsewhere; relevant data illustrating their comparable status relative to grouping are given in Table 1.

Jejunal mucosal biopsies were obtained under fluoroscopic control from the jejunum immediately distal to the ligament of Treitz with a Crosby capsule. After retrieval, each specimen was quickly spread out and orientated, fixed in 10% formal–saline (pH 7.2) for 24 hours, embedded in paraffin wax, sectioned at 4 μ of thickness, and stained with hematoxylin and eosin (H&E).

Quantitative Methods

Each specimen was classified subjectively by one observer (M.M.) to be normal or to show mild, moderately severe, or severe grades of mucosal abnormality (Table 1), as described previously. For quantitative morphometric analysis (M.N.M.), sections were examined through an oil-immersion objec-

Table 1 – Characteristics of the Different Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Age (range)</th>
<th>Hemoglobin g/l (± SD)</th>
<th>Serum albumin g/l (± SD)</th>
<th>Xylose</th>
<th>Fecal fat</th>
<th>B12 absorption</th>
<th>Biopsy grade</th>
<th>N</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>8</td>
<td>25-49</td>
<td>11.6 (2.2)</td>
<td>4.2 (0.6)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II. Epidemic sprue</td>
<td>7</td>
<td>38-50</td>
<td>10.3 (2.5)</td>
<td>3.8 (1.0)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>III. Endemic sprue</td>
<td>11</td>
<td>20-68</td>
<td>8.6 (1.8)</td>
<td>2.7 (0.8)</td>
<td>11</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IV. Recovered from sprue</td>
<td>4</td>
<td>20-50</td>
<td>11.8 (2.7)</td>
<td>4.0 (0.6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Normal values: Fecal fat less than 5 g/day. O-xylene more than 20% of 5 g dose in 5-hour urine. Vitamin B12 absorption more than 0.2% oral dose per liter in plasma.

tive (×100), and measurements were made with reference to a calibrated ocular graticule (Graticules Ltd., Tonbridge, Kent, U.K.) as detailed elsewhere. All quantitative analyses were carried out "blindly"; and once the diagnostic category of each specimen was known, no recalculation or modification of the data took place.

**Determination of Areal Density (AD)**

The number of lymphocytes within surface, and crypt, epithelium was determined for every specimen with reference to a constant test area of muscularis mucosae with dimensions 1000 μ in length and 100 μ in width (Figure 1). In practice, the ocular graticule (1000 μ in length) was oriented parallel to the muscularis mucosae, and the number of lymphocytes contained within surface, and crypt, epithelium overlying the grid was counted. By realigning the 1000-μ grid parallel to muscularis in another region of the same, or succeeding sections, the count was repeated until the total number of epithelial lymphocytes overlying the 1000 × 100-μ test area of muscularis mucosae was obtained.

The number of applications of the grid required to obtain the complete and correct count was determined by the mean nuclear profile diameter (NPD) of epithelial lymphocytes (see below) and hence by the "effective section thickness" (T). The latter was calculated for each specimen from the relationship $T = t + d$, where $t$ is actual section thickness (ie, 4 μ) and $d$ is the mean NPD. The number of epithelial lymphocytes contained within a volume of epithelium is not simply the number of profiles observed in a series of sections, because each "profile" represents a fraction of the whole structure. It has been shown that all the particles (ie, nuclei) of radius $t$, whose profiles appear in a section of finite thickness, $t$ (ie, 4 μ), are contained within a "superslice" of thickness $t + 2t$. In other words, all nuclear profiles observed in a section of thickness $t \mu$ have their centers within the superslice; and, conversely, those profiles whose centers lie without the superslice do not exhibit profiles in section $t$ (Figure 2). The thickness of the superslice within which all the observed profiles lie is termed the effective section thickness (T).

Thus, if the mean NPD (2t) of lymphocytes is 10 μ, then the width of the superslice $T = (4 + 10) = 14 \mu$. Hence the number of applications of the 1000-μ grid necessary to enumerate all profiles within the surface, and crypt, epithelium overlying the total 1000 × 100-μ² rectangle of muscularis mucosae would be 100/14 = 7 separate applications. (If actual section thickness were used in this calculation, ie, 100/4 = 25 applications, the accumulated total cell count would be grossly overestimated.)

For purposes of presentation, we prefer to express the results in terms of 100² sq μ of muscularis mucosae. Since 10 × this area was analyzed in this study, we subsequently divided the counts by 10 in order to relate the number of lymphocytes within each compartment (epithelial and crypt) to the chosen area of muscularis. This procedure, while allowing a greater volume of mucosa to be sampled, reduced by 1/10 the counting errors inherent in the technique. From these data, the total lymphoid pool (crypt AD +

**Figure 1** - Lymphocytes are counted in surface and crypt epithelium overlying a test area of muscularis mucosae 1000 μ long and 100 μ wide. The graticule occupies the long side of the test area, and lymphocytes in epithelium overlying 1000 μ of muscularis mucosae are counted. The number of applications of the grid to other sections necessary to find the total number of lymphocytes overlying the test area is determined by dividing the width of the test area (100 μ) by effective section thickness (see Figure 2).
surface AD), and the percentage of crypt AD to total lymphoid pool, were calculated.

**Nuclear "Profile" Diameter of Epithelial Lymphocytes**

The sectioned, or "profile" nuclear diameters of 200 villous (or surface) and of 100 crypt epithelial lymphocytes were measured for each specimen, and their means were calculated. In addition, the percentage of immunoblastoid ("blast-transformed") lymphocytes within both measured populations per specimen was determined. For this purpose, an immunoblast was arbitrarily defined as a lymphocyte with NPD >6 μ (Figure 3); as previously shown, 3 such nuclei are bounded by a dense rim of chromatin and contain numerous densely staining nucleoli within an expanded area of paler-staining euchromatin.

**Mitotic Index (%)**

We counted all metaphase-anaphase figures in a total count of 3000 lymphocytes within the surface epithelium of each specimen and hence determined the mitotic index (%). Care was taken to avoid epithelium adjacent to crypt mouths, especially in severe lesions, where, by analogy with celiac sprue, 15,16 mitotic epithelial cells are likely to be present. Any doubtful mitotic figure was not counted. Similar determinations were not performed on crypt epithelium because of the impossibility of distinguishing between epithelial and lymphocyte mitoses.

**Flux Ratio**

Lymphocytes positioned within the epithelial basement membrane were considered to be moving either

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**Figure 2**—These micrographs illustrate large nuclei (diameter >6 μ) of immunoblasts (BL) within epithelium of tropical sprue mucosa.  

- a—Control mucosa, mild lesion.  
- b—Epidemic sprue, moderate lesion.  
- c—Endemic sprue, severe lesion. (H&E, x 130)
into the epithelium from the lamina propria, or vice versa. All lymphocytes thus positioned (Figure 4) were counted, and the ratio to their respective surface epithelial AD was determined for the flux ratio (FR) for surface epithelium.

Statistical Analysis of Data

Results shown in Tables 2-4 and Figure 6 were analyzed by unpaired $t$ tests.

Data in Table 5 were analyzed in a non-parametric test (Spearman correlation coefficient): all $t$ tests were two-tailed, because it could not be assumed that each variable necessarily increased with time.

In order to study net changes within this set of data, all three variables (the percentage of crypt immunoblasts, the percentage of surface epithelial immunoblasts, and the mitotic index) were analyzed simultaneously. A "weighted factor" for each vari-

Figure 4—This micrograph illustrates lymphocytes (arrows) in transect across the basement membrane (BM) between the epithelium and lamina propria. The flux of lymphocytes across the basement membrane was obtained by calculating the ratio of such penetrating cells to the number of lymphocytes within the epithelium relative to $10^4$ sq $\mu m$ of muscularis mucosae. (H&E, $\times1300$)

Figure 5—Representative micrographs of crypt sections from control subjects (a) and patients with epidemic (b) and endemic (c) tropical sprue. An example of the focal nature of lymphocytic infiltration of crypt epithelium is shown in c. (H&E, $\times70$)
Table 2—Lymphocyte Areal Densities and Flux Ratios

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Epidemic sprue</th>
<th>Endemic sprue</th>
<th>Recovered endemic sprue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Areal density (per 100 sq μm area of muscularis mucosae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Total lymphocytes</td>
<td>514 ± 117</td>
<td>556 ± 141</td>
<td>463 ± 165</td>
<td>521 ± 171</td>
</tr>
<tr>
<td>ii) Surface epithelial component</td>
<td>440 ± 112</td>
<td>443 ± 130</td>
<td>328 ± 127</td>
<td>444 ± 185</td>
</tr>
<tr>
<td>iii) Crypt component</td>
<td>74 ± 23(1)</td>
<td>113 ± 23(4)</td>
<td>157 ± 64(3)</td>
<td>77 ± 22(3)</td>
</tr>
<tr>
<td>iv) % Crypt to total</td>
<td>14.95 ± 4.73(1)</td>
<td>21.06 ± 5.18(3)</td>
<td>33.0 ± 9.5(3)</td>
<td>17.99 ± 9.63(3)</td>
</tr>
<tr>
<td>Flux Ratios (surface epithelium only)</td>
<td>0.013 ± 0.004(4)</td>
<td>0.018 ± 0.007(4)</td>
<td>0.022 ± 0.007(3)</td>
<td>0.017 ± 0.007(3)</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Total lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii) Epithelial components</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iii) Crypt component</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv) % Crypt to total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flux Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Differences between groups in category i and ii were NS.
y = 0.1833 (crypt) + 0.162 (surface) + 2.988 (micron index)
All values of y were plotted against time, and the line of best fit was drawn by computer. The Spearman (nonparametric) correlation with time was then obtained, and its significance was determined by a two-tailed t-test. (These analyses were performed by Dr. R. D. Baker, Laboratory of Computational Science, University of Salford.)

Results

Areal Density

There was no significant difference in either the total lymphoid population (crypt AD + surface AD) or the surface epithelial lymphocyte population (surface AD) between any group of patients (Table 2). The values of crypt AD were, however, significantly higher in both groups of sprue patients, compared with the control and recovered groups. Moreover, the ratio of crypt to total lymphoid AD (%) was significantly greater between the control and epidemic sprue groups and between the epidemic and endemic sprue groups (Table 2).

The lymphocytic infiltration of the crypt epithelium, as documented quantitatively for each group of sprue patients, was not uniformly distributed, but focal (Figure 5). Only some crypt profiles within each section showed clustering of lymphocytes, which were predominantly confined to the upper one-third of the crypt epithelium and that of the crypt-villus interzones (Figure 5).

Mean Epithelial Lymphocyte NPD

The mean epithelial lymphocyte NPD of crypt lymphocytes in epidemic tropical sprue patients (Group II) was significantly higher than that of control subjects, while that for surface lymphocytes was not (Table 3). The NPD of both surface and crypt lymphocytes in the chronic endemic tropical sprue groups (Group III) were significantly greater than those of control subjects. There was no significant difference in NPD between the two groups of sprue patients. The NPD of recovered sprue patients (Group IV) was similar to that of control subjects and significantly smaller than that of chronic sprue patients (Table 3).

Table 3—Mean Nuclear Profile Diameter of Epithelial Lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Epidemic sprue (≤1 month's duration)</th>
<th>Endemic sprue (&gt;6 month's duration)</th>
<th>Recovered from endemic sprue (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 7)</td>
<td>(n = 9)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>Surface epithelium (mean ± SD)</td>
<td>4.15 ± 0.28 μm</td>
<td>4.43 ± 0.22 μm</td>
<td>4.58 ± 0.23 μm</td>
<td>4.12 ± 0.095 μm</td>
</tr>
<tr>
<td>Crypt epithelium (mean ± SD)</td>
<td>4.13 ± 0.25 μm</td>
<td>4.46 ± 0.24 μm</td>
<td>4.55 ± 0.14 μm</td>
<td>4.07 ± 0.21 μm</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>a vs b, NS</td>
<td>a vs c, P &lt; 0.005</td>
<td>a vs d, P &lt; 0.005</td>
<td>a vs e, P &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>a vs f, P 0.05</td>
<td>a vs g, P &lt; 0.005</td>
<td>g vs h, P &lt; 0.001</td>
<td>g vs i, P &lt; 0.001</td>
</tr>
</tbody>
</table>


Table 4 — Percentage of Immunoblasts Within Surface and Crypt Epithelium

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 8)</th>
<th>Epidemic sprue (n = 7)</th>
<th>Chronic endemic sprue (n = 11)</th>
<th>Recovered from endemic sprue (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface epithelium (mean ± SD)</td>
<td>1.0 ± 0.96(8)</td>
<td>5.71 ± 3.51(8)</td>
<td>6.7 ± 5.5(8)</td>
<td>1.2 ± 0.64(8)</td>
</tr>
<tr>
<td>Crypt epithelium (mean ± SD)</td>
<td>0.87 ± 0.89(8)</td>
<td>4.14 ± 3.16(8)</td>
<td>6.7 ± 3.7(8)</td>
<td>0.25 ± 0.9(8)</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface epithelum</td>
<td>a vs b, P = 0.005</td>
<td>a vs c, P &lt; 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crypt epithelum</td>
<td>e vs f, P = 0.02</td>
<td>e vs g, P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage of Epithelial Immunoblasts and Mitotic Index

In both groups of tropical sprue patients the percentage of immunoblasts within crypt and surface epithelium was significantly greater than that of controls (Table 4). The percentage of immunoblasts in the recovered sprue patients was reduced and was not significantly different from that of the control group.

The mitotic activity of control epithelial lymphocytes was minimal except for 2 patients (Figure 6). In both groups of tropical sprue patients, values for mitotic index varied widely; some values were markedly raised, while many fell within the control range. The mitotic index of 3 of the 4 recovered sprue patients also lay within the control range (Figure 6).

In patients with epidemic sprue, there was an upward trend in indexes of lymphocyte “activation” (i.e., the percentage of immunoblasts within surface and crypt epithelium and the mitotic index) with duration of illness, although a significant correlation was observed only for surface epithelial immunoblasts (Table 5). However, simultaneous analysis of all three variables (Figure 7) revealed a highly significant correlation between each combined first principal component (y) per patient and time (Spearman coefficient).

Flux Ratios

Flux ratios for lymphocytes within the surface epithelium of control and of epidemic sprue patients were not significantly different (Table 2). In patients with chronic endemic sprue, the FR for surface epithelium was significantly higher in comparison with controls but did not differ significantly from that of acute epidemic sprue patients.

Discussion

These data, which were all obtained “blindly,” without knowledge of the diagnostic category, clearly establish that an “immunologic component” of the mucosal lesion of Indian tropical sprue is demonstrable within crypt and surface epithelium. These

Figure 6 — The percentage of mitotic indexes of surface epithelial lymphocytes is significantly raised, both in epidemic (P = 0.06) and endemic (P < 0.02) tropical sprue patients, compared with control subjects.
changes were shown with morphometric techniques in an analysis of epithelial lymphocytes, which exhibited alterations both in structure and number, compared with control and recovered tropical sprue mucosae. Our data do not rule out concurrent involvement of the lamina propria and what role the latter might play in triggering the epithelial lymphocyte abnormalities described herein or the epithelial cell dysfunction.

This study revealed that alterations in the number, size, mitotic activity, and degree of blast transformation of epithelial lymphocytes only became detectable after symptoms had been present for 2 or more weeks. In view of these observations, the appearance and extent of various indices of epithelial lymphocyte “activation” (Table 5) in relation to the duration of illness in patients with epidemic sprue (<30 days) were evaluated statistically. No significant correlation between mitotic activity and duration of illness was established. However, the trend toward a higher percentage of mucosal immunoblasts at the period of illness lengthened was statistically significant (r = 0.9141; P < 0.005) for blasts within surface epithelium, although a similar correlation for crypt immunoblasts failed to achieve levels of significance. However, when each set of data per person was linearly correlated (Figure 7) a highly significant relationship with the duration of disease became apparent. It was thus concluded that the “activation” of epithelial lymphocytes was likely to be interrelated and probably triggered by the same mechanism.

Because there was no detectable change in mucosal epithelial lymphocytes in patients symptomatic for less than 5 days, but in whom malabsorption and moderately severe intestinal morphologic lesions were already established, it was concluded that the lymphocyte abnormalities documented in the remaining sprue patients could not be etiologically relevant to their disease, but only secondary to disease which had already affected enterocyte function and mucosal architecture.

Another important conclusion of this study con-
cerns changes in mucosal lymphocyte densities observed in both groups of patients relative to control subjects. Here the results directed attention not solely to the epithelium (as in celiac sprue and cow's milk hypersensitivity) but particularly to the crypts as the site of major immunologic change. The data revealed increased numbers of lymphocytes within crypt epithelium only, whether expressed as crypt AD or as a percentage of total epithelial lymphocytes (Table 2); there was no change in the density of lymphocytes within the surface epithelial compartment of either epidemic or endemic sprue mucosa.

The patchy or focal distribution of the lymphoid infiltrates observed histologically (Figure 5) within crypt epithelium suggested that these were related to the disease process and not merely a reflection of crypt hypertrophy, in which case they would have been distributed more evenly. Whether the severity of the mucosal lesion is directly related to the number of infiltrated crypts is not known and was not pursued in this study. These results, and previous observations from this laboratory, lead to the conclusion that the crypts appear to be the predominant site of epithelial injury, and that the damaged surface cells are secondary to this process.

The striking difference between tropical sprue and celiac sprue is clearly brought into focus by this study. In gluten sensitivity, the brunt of the disease falls upon the surface (villous) epithelium, which may be chronically exposed to gluten for several years, and in which there is extensive damage to enterocytes. Furthermore, as distinct from tropical sprue, the cells in the upper crypt regions in untreated celiac sprue are entirely normal, as shown by careful histochemical and transmission electron microscopic studies; abnormalities only occur as emergent enterocytes gain the surface epithelium. There is no evidence of a primary crypt lesion in celiac sprue; neither are the crypts the site of lymphoid infiltration, unlike those in tropical sprue (Figure 5). Gluten challenge evokes synchronous morphologic and immunologic changes within surface epithelium and lamina propria within 4–48 hours, followed by complete restoration of mucosal integrity. Thus, while immunologic abnormalities parallel, and may initiate, tissue damage in celiac sprue, the evidence presented here clearly shows that immunologic involvement in tropical sprue is secondary and thus unlikely to play any primary role in its pathogenesis.

The accumulation of "chronic inflammatory" cells in the jejunal epithelium of patients with tropical sprue in India and the Caribbean was described in earlier studies. These infiltrating cells, many of which are now recognized as the functional agents of local immunologic events within the intestinal epithelium and lamina propria, were not quantitated. In the few studies where epithelial lymphocytes have been quantitated in the surface epithelium (per 100 epithelial cells) both in tropical sprue and celiac sprue, an increase in lymphocyte numbers was interpreted to reflect local cell-mediated activity. However, when epithelial lymphocytes in celiac sprue were quantitated by the AD method, they were somewhat fewer in number than in control biopsies, although other parameters of lymphocyte activation, eg, percentage of immunoblasts, mitotic indexes, and flux ratios, showed that there was, in fact, an "immunologic" abnormality. Changes in the number of epithelial cells may influence the lymphocyte count, and the variable amount of epithelial cell loss explains the apparent discrepancies between different studies on epithelial lymphocyte quantitation.

The "areal density" method of lymphocyte quantitation, with the additional morphometric parameters of lymphocyte "activation," would appear to be a more sensitive technique for studying epithelial lymphocytes irrespective of alterations in epithelial cell mass.

The increase in immunoblasts in the surface epithelium (Table 4) was not significantly different between patients with epidemic and endemic sprue, and in the latter group was not related to the duration of symptoms. The majority of intestinal epithelial lymphocytes are cytolytic and suppressor T-lymphocytes, and epithelial immunoblasts are ultrastructurally identical to blasts produced by either in vitro and in vivo stimulation. Evidence of lymphocyte activation in the epithelium of a gluten-sensitive patient with severe hypogammaglobulinemia is strong evidence that these activated cells are T lymphocytes. The lymphocyte activation in celiac sprue, which returns to normal on gluten withdrawal, is probably mediated by blastogenic and mitogenic lymphokines generated by a local cell-mediated immune response to gluten. The genesis of lymphocyte activation in the jejunal epithelium in tropical sprue can be understood only after the causative agent(s) of the mucosal lesion is known.

Raised lymphocyte mitotic index in the surface epithelium provides a marker of celiac sprue that prospectively distinguishes it from other malabsorption syndromes accompanied by a flat, or severely damaged, mucosa. The changes in the mitotic indices in the present study were not so clear-cut and probably reflect the basic difference in the primary site of the mucosal lesion in tropical sprue (crypt) and celiac sprue (surface epithelium).
The changes in flux ratios were also less marked in this group of patients with tropical sprue and may be a reflection of the less severe mucosal morphologic lesion. The rate of loss of enterocytes\textsuperscript{18, 19} and lymphocytes\textsuperscript{20} in celiac sprue is high, and the latter is probably responsible for the higher lymphocyte flux ratios. Dynamic studies of intestinal epithelial lymphocyte turnover are difficult,\textsuperscript{21, 22} but the flux ratio, a static measurement of a dynamic process, probably is an indication of increased lymphocyte traffic across the basement membrane of these enterocytes.

The immunologic alterations in the intestinal epithelium in celiac sprue are related to the etiologic agent. The data presented here show that "activation" of epithelial lymphocytes in tropical sprue occurs subfrequent to enterocyte damage, is more marked within crypt epithelium, does not progressively increase in chronic patients, and becomes normal on recovery. These results show that the immunologic alterations are unlikely to be a primary event damaging the enterocyte, although they might have a role in the perpetuation of the mucosal lesion. Their full significance can only be understood when the etiology of the syndrome of tropical sprue is known.

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