Geographical Variations in the Morphology of the Small Intestinal Mucosa in Apparently Healthy Individuals

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Abstract. Macroscopic and microscopic photographs of seven jejunal biopsies illustrating the range of morphological changes found in southern Indian control subjects were circulated, together with a questionnaire, to observers in different parts of the world. There was general agreement among the observers regarding the 'normality' of the biopsy showing the least changes and the abnormality of the two showing the most marked changes, but considerable disagreement over those intermediate between these two extremes. Opinion regarding the prevalence of the different biopsy appearances in control subjects varied significantly between temperate and tropical areas. A hierarchy of abnormality has been drawn up ranking countries in decreasing order of prevalence of the two most abnormal biopsies. Possible factors in the pathogenesis of the morphological changes in the jejunal mucosa in the tropics are discussed.

A number of investigators working in the tropics have found that small intestinal biopsies from apparently healthy individuals may show changes that, in temperate areas, would be associated with overt gastrointestinal disease [Baker et al., 1962; Sprinz et al., 1962; Banwell et al., 1964; Russell et al., 1966; Lindenaum et al., 1966; Klipstein et al., 1966; England and O'Brien, 1966; Robins et al., 1967; Troncale et al., 1967; Halstead et al., 1969; Cook et al., 1969].

Because of the difficulties of quantitation, different observers appear to have used differing criteria for the 'normality' or otherwise of both the macroscopic and microscopic appearance of biopsies. Some investigators have measured the height and width of villi as they appear in section.

Key Words

Jejunal mucosa
Jejunal biopsy
Villus architecture
Crypt-villus ratio
Tropical enteropathy

1 Wellcome Trust in association with World Health Organization.
However, these measurements, and the general appearance of the biopsy, are influenced by a variety of factors such as the state of contraction or relaxation of the smooth musculature of the villus and (when present) of the muscularis mucosae; the direction in which the block is sectioned; the consistency of the embedding material; the room temperature; the sharp-
ness of the knife; the thickness of the section and the angle at which the section is cut. Rouns et al. [1960] have emphasised the importance of sections being cut vertical to the serosal surface in order to get an adequate appreciation of crypt height and villus structure. The literature is unfortunately full of statements made about biopsies which have not been sectioned vertically, and which consequently do not give an adequate representation of the morphology. When the villi are leaf or ridge shaped the angle at which they are cut, compared with the long axis of the villi, will also produce marked differences in the apparent width of the villi [Baker et al., 1962]. Unless sectioned in their minimum diameter, any statement about the villus 'width' and any attempt at quantitation of the mucosal surface is valueless. To produce comparable results it is clearly necessary to closely standardise embedding and cutting conditions. Until this is done a meaningful comparison of biopsies from different centers is difficult or impossible.
This study was undertaken to try and determine firstly what different workers considered to be 'normal' intestinal biopsy findings and, secondly, the prevalence with which biopsies, of differing degrees of abnormality, occurred in apparently healthy people in temperate and tropical regions. To overcome the lack of comparability between different centers, questionnaires (appendix) were prepared on seven macroscopic and microscopic biopsy photographs (fig. 1–7). These were sent to 40 investigators, in different parts of the world, who were known to be interested in the subject. The biopsies were taken from the first loop of jejunum of asymptomatic subjects who gave their informed consent to the study. All had normal fat, vitamin B₁₂, and xylose absorption. The biopsies were serially sectioned at 4 μm, stained with haematoxylin and eosin and a typical section photographed. The replies received have been analysed and the results are given below. The test of statistical significance used in the analysis was the \( \chi^2 \) test.
Replies were received from 34 investigators, of whom 30 completed the questionnaires. Some individuals had had experience in more than one region so that there were a total of 42 observer-situations, 19 in temperate regions and 23 in the tropics. Several observers were understandably reluctant to give an opinion on the basis of a photograph of one section, but nevertheless were kind enough to cooperate. Not all observers answered all the questions – though no question was answered by less than 92% of observers.

**Villar Architecture**

The percent of observers who consider the dissecting microscopic appearance of each of the biopsies to be normal is shown in table I. There are some differences between observers in temperate and tropical regions but these do not attain statistical significance. Biopsy B (fig. 2) consisting mainly of narrow leaves, is considered normal by all except three observers, whereas biopsies C and E (fig. 4, 6) which have convoluted patterns, are considered abnormal by nearly all observers.

The opinions regarding the prevalence of each type of biopsy in ‘apparently healthy subjects without evidence of malabsorption’ in temperate zones and in the tropics is shown in figure 8. The results for biopsies A, D and G and for biopsies C and E are practically identical and are, therefore, given together. The narrow leaf-like villi of biopsy B are considered to occur in more than 25% of normal subjects by 93% of the observers from temperate zones, whereas 39% of observers from the tropics consider that this villus form never occurs or is seen in 25% or less of normal subjects (p < 0.065). The convoluted pattern of biopsies C and E is thought by 84% of temperature zone observers never to occur or to occur in less than 5% of normal subjects, whereas in the tropics 56% of observers find it in 5% or more of normal subjects (p < 0.005).

**Histology**

The percent of observers who consider the histological sections of the biopsies to be normal is shown in table II. There is no significant difference between temperate and tropical regions. Biopsy B (fig. 2) was considered normal by all except two observers (95%). At the other extreme, biopsy E (fig. 5) was considered normal by only one observer from the tropics. The opinions regarding the normality of specific histological features are also similar for temperate and tropical regions and are shown combined in table III.

The prevalence of these histological appearances in apparently healthy subjects in the temperate and tropical regions is shown in figure 9. The results for biopsies D, F and G and biopsies C and E are practically identical and are, therefore, given together. An appearance similar to biopsy B is found in 75-100% of healthy subjects in the temperate zones in the experience of 53% of the observers, but in the tropics only 10% of the observers place it in this category (p < 0.005). Indeed, 10% of observers in the tropics are of the opinion that a biopsy picture like this is never found in normal subjects. Biopsies D, F and G have a similar pattern of prevalence in both temperate and tropical areas. An appearance similar to biopsy A
Table I. The percentage of observers who consider the dissecting microscopic appearances of biopsies A–G to be normal

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Temperate</th>
<th>Tropics</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>95</td>
<td>91</td>
<td>93</td>
</tr>
<tr>
<td>F</td>
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<td>41</td>
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<td>G</td>
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<td>27</td>
<td>27</td>
</tr>
<tr>
<td>D</td>
<td>16</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>A</td>
<td>11</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>C</td>
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<tr>
<td>E</td>
<td>0</td>
<td>5</td>
<td>2</td>
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</tbody>
</table>

Table II. The percentage of observers who consider the histological appearances of biopsies A–G to be normal

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Temperate</th>
<th>Tropics</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>100</td>
<td>91</td>
<td>95</td>
</tr>
<tr>
<td>D</td>
<td>68</td>
<td>57</td>
<td>62</td>
</tr>
<tr>
<td>F</td>
<td>53</td>
<td>64</td>
<td>58</td>
</tr>
<tr>
<td>G</td>
<td>53</td>
<td>59</td>
<td>56</td>
</tr>
<tr>
<td>A</td>
<td>11</td>
<td>22</td>
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</tr>
<tr>
<td>C</td>
<td>0</td>
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</tr>
<tr>
<td>E</td>
<td>0</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Table III. Percent of observers who consider the findings to be normal in each biopsy. The remainder consider them to be either abnormal or are uncertain. Data from temperate and tropical regions are similar and are combined in this table

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>B</th>
<th>D</th>
<th>G</th>
<th>F</th>
<th>A</th>
<th>C</th>
<th>E</th>
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</thead>
<tbody>
<tr>
<td>Villus height</td>
<td>100</td>
<td>100</td>
<td>95</td>
<td>67</td>
<td>43</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Crypt height</td>
<td>100</td>
<td>98</td>
<td>90</td>
<td>98</td>
<td>14</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Villus width</td>
<td>83</td>
<td>79</td>
<td>69</td>
<td>52</td>
<td>45</td>
<td>17</td>
<td>5</td>
</tr>
<tr>
<td>Lamina propria cellularity</td>
<td>76</td>
<td>57</td>
<td>31</td>
<td>78</td>
<td>31</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Epithelial cellularity</td>
<td>88</td>
<td>62</td>
<td>55</td>
<td>67</td>
<td>57</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>100</td>
<td>93</td>
<td>83</td>
<td>78</td>
<td>55</td>
<td>43</td>
<td>7</td>
</tr>
</tbody>
</table>
Fig. 8. Histogram showing the percent of observers (ordinate) in temperate areas (open blocks) and tropical areas (stippled blocks) who place the dissecting microscopic appearance of the biopsy in one of five categories of prevalence (abscissa); never occurs N, occurs in 0-5, 5-25, 25-75, 75-100% of normal subjects.

Fig. 9. Histogram showing the percent of observers (ordinate) in temperate areas (open blocks) and tropical areas (stippled blocks) who place the histological appearance of the biopsy in one of five categories of prevalence (abscissa); never occurs N, occurs in 0-5, 5-25, 25-75, 75-100% of normal subjects.
<table>
<thead>
<tr>
<th>Country</th>
<th>Score</th>
<th>Region</th>
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</thead>
<tbody>
<tr>
<td>Haiti</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vietnam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haiti</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Columbia</td>
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<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td></td>
<td></td>
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<tr>
<td>South Africa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
<td>UK</td>
</tr>
<tr>
<td>Australia</td>
<td>0</td>
<td>Germany</td>
</tr>
<tr>
<td>Belgium</td>
<td></td>
<td>Belgium</td>
</tr>
<tr>
<td>India, Punjab</td>
<td>4</td>
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</tr>
<tr>
<td>India, Vellore</td>
<td></td>
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<tr>
<td>Nigeria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singapore (Indians)</td>
<td></td>
<td>USA</td>
</tr>
<tr>
<td>India, Madras</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>India, Punjab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td></td>
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</tr>
</tbody>
</table>

is not thought by anyone to occur in more than 25% of normal subjects in temperate areas, but in the tropics 40% of observers find it in 25-100% of normal subjects (p < 0.025). Appearances similar to biopsies C and E are considered never to occur or to occur in less than 5% of normal people in temperate zones by 94% of observers, whereas 58% of observers in the tropics are of the opinion that such biopsies may be found in more than 5% of healthy subjects (p < 0.0005).

In order to compare different areas, the answers given to the questions regarding the prevalence of the histological appearances of biopsies C and E were given a score as follows: never found, 0; occurs in less than 5%, 1; occurs in 5-25%, 2; occurs in 25-75%, 3; and occurs in more than 75%, 4. The scores for the two biopsies were added and the results ranked in order of descending score, thus, establishing a 'hierarchy of abnormality' for the different countries (table IV). The higher a country is in the hierarchy, the more prevalent are the abnormal histological appearances in apparently healthy subjects without evidence of malabsorption.
Normal Intestinal Morphology

It would seem desirable to define 'normal' intestinal morphology as that which is found in healthy individuals, living under optimal conditions of nutrition and hygiene, who have no evidence of malabsorption. As such the normal appearance would not be expected to differ in different parts of the world unless there were genetically determined variations. The fact that there is no significant differences between replies from tropical and temperate regions indicates that the concept of normality used by the majority of observers is independent of the prevalence of a particular finding in the community. However, a few observers, with experience in both temperate and tropical areas, considered some appearances normal in the tropics but abnormal in temperate regions, indicating that their concept of normality was influenced by prevalence. There is almost complete agreement over the classification of both macroscopic and microscopic pictures of biopsy B, on the one hand, as being 'normal' and biopsies C and E, on the other, as being 'abnormal'. There is, however, much less agreement on the other four biopsies A, D, F, and G and all possible permutations and combinations of opinions are expressed in regard to those biopsies, emphasizing the inevitable subjectivity of morphological judgements and the difficulty of defining minor degrees of abnormality.

If the opinion of 50% or more of the observers is arbitrarily taken as defining 'normality' then the dissecting microscopic appearance of biopsy B and the histological appearances of biopsies B, D, G and F can be considered to be within normal limits.

Prevalence of Abnormality

The prevalence with which biopsies C and E are considered to occur in apparently healthy subjects differs significantly in temperate and tropical areas, thus confirming the previous reports from different parts of the tropics. The hierarchy of abnormality, based on the opinions regarding biopsies C and E, for the first time permits some quantitation of the prevalence of abnormal findings in different regions. There are several apparent inconsistencies in this list: the two observers who studied Indians in Singapore (scores 4 and 0), the observer in USA who gave a score of 4 in contrast to the four who gave a score of 0; the observer from Puerto Rico who gave a score of 1 as compared with the two who gave a score of 6 and the observer from Western India who gave a score of 0, whereas
other Indian observers gave scores of 4 or 3. These may represent true intracountry variations in biopsy appearances or, more probably, they may be the result of observer variation.

Aetiology

The aetiology of these biopsy abnormalities in apparently healthy subjects living in many parts of the tropics is unknown. KLIPSTEIN [1967] has suggested they may be related to the lesion of tropical sprue. In those areas where sprue is prevalent, the changes found in some individuals may be of the same aetiology as tropical sprue. However, present epidemiological evidence does not support the concept that the lesions in the majority of individuals are so related [Baker and Mathan, 1972].

In a previously reported study CHACKO et al. [1968] showed that human fetuses in southern India have finger-like villi, but that these change within the first few weeks of life to leaf-shaped villi and later, in many, to ridges or convolutions. In other words, the changes appear in some way to be related to exposure to the environment. This is further borne out by the fact that expatriates moving to tropical countries tend to develop changes similar to those seen in the local population [LINDENBAUM et al., 1966; SHEEHY et al., 1968] which regress on returning to temperate climates [SHEEHY et al., 1968]. The changes are more marked proximally than distally [CHACKO et al., 1969; WALKER-SMITH, 1972] and are more prone to occur on the crests of the valvulae conniventes than in the hollows [CREAMER and LEPPARD, 1965] suggesting that areas most exposed to trauma are more likely to be affected. Experimental studies in rats show that surgical exclusion of a loop of jejunum results in halting or regression of these changes [CHACKO et al., 1968; GLEESON et al., 1970] suggesting that the continued passage of chyme is important and that some toxic factor in the diet may play a part in their aetiology. However, no such toxic factors have yet been identified either in animals or man.

Inspection of the hierarchy of abnormality (table IV) indicates that, with some exceptions, similar ranking could be assigned for factors such as intestinal parasitism, environmental contamination and malnutrition and suggests that these may play a role in the production of the abnormality.

It has been claimed that infestation with hookworm may cause morphological changes in the intestine [SHEEHY et al., 1962; TANDON et al., 1966; CHUTTANI et al., 1967]. However, the evidence is conflicting [LAYRISSE et al., 1964; BURMAN et al., 1970] and further controlled studies are
needed in this area. Infestation with other parasites such as *Strongyloides stercoralis* [Stemmermann, 1967], *Giardia lamblia* [Yardley et al., 1964] and *Capillaria philippinensis* [Whalen et al., 1969] may cause changes in the intestinal mucosa, but the distribution of these parasites is not sufficiently ubiquitous to be responsible for the observed abnormalities.

In germ free animals, the villi are taller, the crypts shorter and the lamina propria less cellular than in conventional animals [Abrams et al., 1963], but the role of bacteria in producing changes in intestinal morphology in man is not clear. The upper jejunum of subjects living in temperate regions is usually sterile or has less than 10^5 organisms per ml of jejunal juice [Creagan and Hayward, 1953; Bornsrod and Cohn, 1965; Gorbach et al., 1967; Drasar et al., 1969]. Very few adequate studies of the luminal flora of the jejunum of normal subjects in the tropics have been carried out. In southern Indian control subjects, Bhat et al. [1972] found 8 out of 16 subjects with bacterial counts of 10^6 or more per ml of jejunal juice. It is possible that this bacterial contamination may produce alterations in the morphology of the intestinal mucosa. However, bacteria are invariably more numerous in the more distal regions of the small intestine where villus changes are less marked. Also, patients in temperate zones with the stagnant bowel syndrome have large numbers of luminal bacteria, but in general, are reported to have normal intestinal biopsies [Donaldson, 1965]. The possible role of bacterial contamination of the intestinal lumen in producing the observed histological changes is, therefore, very difficult to assess.

The role of viruses, if any, in producing changes in intestinal morphology is also not clear. Subjects living in tropical areas are exposed to a wide variety of viral agents. For example, viral isolates can be obtained from 58% of randomly collected faecal specimens from south Indian children [John, 1972]. It is possible that infection with one or more viruses may be responsible for producing alterations in human intestinal morphology, but there is at present no evidence to support or refute this.

The role of nutritional deficiencies is controversial. The changes are seen even in subjects living on a good diet [Baker et al., 1962] so dietary factors cannot be the only cause. Monkeys maintained on a protein-free diet developed some atrophy of both the crypts and the villi but without any changes in the lamina propria [Dio and Ramalingaswami, 1965]. Similar changes were noticed in rats given a protein-free diet for 3 weeks [Takano, 1964]. However, in man the role of protein deficiency is difficult to evaluate. Several studies in children with kwashiorkor have shown
changes in the intestinal morphology which have been attributed to protein deficiency [BURMAN, 1965; STANFIELD et al., 1965; BRUNNER et al., 1966] but these have taken no account of possible gastrointestinal disease as a factor precipitating the kwashiorkor, nor have they taken account of the intestinal morphology of a comparable control group. In adults, several groups of workers [CHUPTANG et al., 1968; TANDON et al., 1968; MAYORAL et al., 1967] have suggested that hypoproteinaemia may produce morphological changes in the intestine, but the interpretation of all these studies is open to question as they were conducted in regions where tropical sprue is common and this may well have been the cause of the hypoproteinaemia and the biopsy changes. Further, patients in temperate areas with hypoproteinaemia due to dietary deficiency [GOUGH et al., 1963], nephrotic syndrome [JENSEN et al., 1966] and liver disease [MARIN et al., 1969] have been found to have normal intestinal biopsies. It, therefore, seems improbable that hypoproteinaemia plays an important role in the pathogenesis of these lesions.

NAIMAN et al. [1964] reported 14 children who had iron deficiency anaemia and abnormal jejunal biopsies. In four children the biopsy was re-examined after therapy and was said to show improvement. However, the possibility that the gut changes and the iron deficiency anaemia may have both been due to some other factor was not adequately explored. RAWSON and ROSENTHAL [1960] studied 10 adults with iron deficiency anaemia and found a normal intestinal biopsy. In 22 subjects with severe iron deficiency anaemia studied in this Unit the intestinal morphology was no different from that of control subjects, suggesting that even severe long standing iron deficiency does not affect the mucosal morphology.

Severe vitamin B₁₂ and folate deficiency will produce minor morphological abnormalities of the intestinal mucosa with macrocytosis of the epithelial nuclei and some degree of crypt hyperplasia [FOROGOZAN and TRIER, 1967; HERMOS et al., 1972]. However, these deficiencies cannot account for the prevalent abnormality which usually occurs even when serum vitamin B₁₂ and folate concentrations are normal.

It must be concluded that the changes in intestinal morphology are the end result of one or more of a variety of as yet unknown insults to the mucosa. Different factors may well be dominant in different regions. The fact that the changes tend to regress only on moving to a temperate zone suggests that exposure to the damaging agent(s) continues while the individual remains in the tropics. Considerably more work needs to be undertaken before the aetiology in different regions can be precisely defined.
Acknowledgements

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Replies were received from the following list of investigators to whom the author is deeply grateful for their help, suggestions and advice.


Appendix

Questionnaire

In the light of your experience of jejunal biopsies from would you consider:

(1) The dissecting microscopic picture to be normal □ abnormal □

In normal subjects (i.e. apparently healthy without evidence of malabsorption) how often would you find such a picture never □ rarely □ occasionally □ frequently □ very frequently □

(2) The histological section to be normal □ abnormal □

In normal subjects how often would you find such a picture never □ rarely □ occasionally □ frequently □ very frequently □

(3) The villus height to be normal □ increased □ uncertain □

(4) The crypt height to be normal □ increased □ uncertain □

(5) The villus width to be normal □ increased □ uncertain □

(6) The cellularity of the lamina propria to be normal □ increased □ uncertain □

(7) The cellular infiltration of the epithelium to be normal □ increased □ uncertain □

(8) The surface epithelium cells (as far as you can tell from this) to be normal □ abnormal □ uncertain □

Any other comments please:

1 Approximate definitions: rarely, less than 5%; occasionally, 5–25%; frequently, 25–75%; very frequently, more than 75%.
References


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