MORPHOLOGIC EFFECTS OF FOLIC ACID AND VITAMIN B_{12} ON THE JEJUNAL LESION OF TROPICAL SPRUE

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Treatment of severe tropical sprue with folic acid and vitamin B_{12} can be dramatically effective and often lifesaving, but usually is not curative. Symptoms, such as diarrhea and abdominal cramps alternating with constipation, flatulent distention of the abdomen, asthenia, and suboptimal recovery of body weight, often remain. After administration of the vitamins, singly or combined, prompt and complete remission of megaloblastic anemia is usually noted. Laboratory tests commonly disclose residual steatorrhea, however, and malabsorption of vitamin B_{12} and xylose as well. Abnormalities of intestinal function may persist for years during continuous folic acid treatment, either alone or with vitamin B_{12},[1] even in dosages considerably greater than the presumed physiologic requirement for man.[2,3]

The microscopic appearance of the jejunum is not significantly abnormal in nutritional folate deficiency uncomplicated by sprue[4] or in the vitamin B_{12} deficiency of pernicious anemia.[5,6] In contrast, the jejunum in untreated folate-B_{12} deficient tropical sprue patients exhibits characteristic abnormalities.[7,8] Treatment with folic acid and vitamin B_{12} may produce improvement in the appearance of the jejunum.[1,9-11] In an endemic area, however, the presence of residual clinical and pathologic evidence of disease even after correction of vitamin deficiencies,[1,6] makes it unlikely that the primary or sole cause of tropical sprue is folate-B_{12} deficiency.

It is unknown to what extent jejunal damage, described in a previous publication as typical of the disease in Puerto Rico,[8] is reversible. To answer this question, a group of previously untreated patients was subjected to a controlled series of observations to determine how the jejunum responds to folate-B_{12} therapy. It was our aim: 1) to provide

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information on the magnitude, time and duration of the morphologic effects of folic acid and vitamin B_{12}, 2) to compare the influence of treatment on mild as contrasted with severe lesions, 3) to specify as clearly as possible the morphologic characteristics of the residual lesion, and 4) to distinguish possible differences effected by these two metabolically interrelated vitamins.

Material and Methods

Jejunal biopsy tissues were obtained from 14 patients with untreated tropical sprue before and after the administration of folic acid or vitamin B_{12}. Seven patients treated only with folic acid, contributed 7 pretreatment and 22 post-treatment specimens. Seven patients treated with vitamin B_{12} only, contributed 7 pretreatment and 17 post-treatment specimens. Each group contained 2 mild, 3 moderate and 2 markedly altered pretreatment specimens. In a previous report [8] we have classified biopsy tissues as borderline, mild, moderate, severe and atrophic. In this paper, we use only three categories, mild (replacing the previous borderline to mild), moderate (equivalent to the previous moderate to moderately severe), and marked (replacing the previous severe to atrophic). Folic acid was administered orally throughout the study (15 mg per day for the first 2 months; then 5 mg per day). Intramuscular vitamin B_{12} was initiated by 30-μg injections given on each of 6 consecutive days, followed by single 30-μg injections once each week. No other therapy was given. The patients received an unrestricted hospital diet. Final biopsy specimens were obtained at 7 to 52 weeks in the folate treated group, and at 26 days to 16 weeks in the vitamin B_{12} treated group. All tissues were obtained with the Crosby-Kugler capsule [12] and fixed in 10 per cent neutral buffered formalin.

The clinical diagnosis of tropical sprue was established by the presence of at least 3 of the 5 most common symptons, i.e., diarrhea, weight loss, weakness, abdominal discomfort and glossitis. The clinical impressions were confirmed by laboratory tests indicating elevated fecal fat excretion, malabsorption of vitamin B_{12}, xylose and vitamin A, depressed serum carotene values, megaloblastic changes in the bone marrow and macrocytic anemia. At least 3 abnormal tests of absorption and an abnormal jejunal biopsy section were recorded for each patient. Both native North Americans and native Puerto Ricans were included. Patients who, in addition to tropical sprue had other diseases or conditions, were excluded.

The equipment and techniques used in the dissecting microscope examination were identical with those described previously.[8] Approximately ½ of each biopsy tissue was set aside and preserved in formalin. Comparisons between these residual fragments were made at the conclusion of the study when all specimens from a single patient could be arranged in chronologic sequence.

Measurements of overall mucosal thickness, obtained from photographs of histologic sections, extended from the bases of the crypts to the villus tips. In each case, 12 to 15 measurements were averaged. An estimation of the epithelial cell population was obtained from sections cut perpendicularly to the mucosal surface by counting the number of cells appearing in an uninterrupted column from the middle of a crypt base to the center of the adjoining villus tip. Ten counts from each specimen were averaged. The relative size of the largest crypt epithelial nuclei was estimated by planimetric measurement of camera lucida tracings drawn at oil immersion magnification. The areas (mm^2) of 50 nuclei per specimen were averaged. In addition, 35 plasma cell nuclei were measured to exclude differences in epithelial nuclear size associated with shrinkage. The number of argentaffin cells per crypt was averaged from a minimum of 50 crypts. Before mitosis counts, focal variation in mitotic activity was assessed at scanning magnification so that zones of high and low

mitotic activity were equalized. Each mitosis index, expressed as the number of mitosis per 100 cells, was based on the number of mitosing cells per 5,000 total cells.

Because of the heterogeneity of the sample, namely, the variability of the initial biopsy fragments, dissimilar timing of post-treatment biopsy, and the different durations of follow-up, the data were useful only in illustrating trends of change in the measured parameters. The treatment response of markedly abnormal specimens, however, was clearly significant. The ranges of the measurements classified in a previous report [8] as normal and nonspecific jejunitis, were used as control values in the present study.

Hematoxylin and eosin stained sections were used for all procedures except argentaffin cell counts where the Fontana-Masson stain was employed. As noted, in all our studies, because of their diminutive size, jejunal biopsy tissues were especially susceptible to heat shrinkage during the preparation of histologic sections. Overheating was an important source of artifact that reduced mucosal thickness and nuclear size. When shrinkage artifact was present, a second piece of tissue from the formalin storage bottle was embedded. Special precautions minimized this hazard: tissues were subjected to infiltration by paraffin (MP 54 —57° C) for not more than ½ hour, and the heated forceps used to place tissue in embedding boats were kept at a temperature that permitted them to be held comfortably against the skin. Paraffin was allowed to congeal at the bottom of the embedding boat, which lowered its temperature and facilitated balancing the tissue in an upright position.

Dissecting Microscope Observations

Each patient's biopsy samples provided a group of specimens in chronologic sequence. Comparison of pretreatment with post-treatment specimens disclosed differences in all cases (Table I). Quantitatively, the extent of change depended on the degree of deviation from normal of the pretreatment sample. Thus, markedly abnormal specimens responded dramatically, moderately abnormal specimens exhibited readily apparent differences, while changes after treatment of mildly affected specimens were subtle.

Changes in villus pattern toward normal, namely, convoluted ridges toward leaves, or leaves toward tongue and finger forms, were slow to develop, variable, and occurred independently of other mucosal effects. For this reason, changes in villus pattern are listed separately from the more rapidly developing responses labeled 'other criteria' (Table I). The latter included: a) reappearance of villi, or, increased height of the existing villi with deepening of the intervillus spaces, b) decreased swelling and crowding, c) increased mobility and resilience, and d) a change in shape of villus tips or crests from flat or rounded to tapered.

In 2 biopsy specimens with mild changes (cases 2 and 8, Table I), the proportion of leaves increased during treatment, this was interpreted as worsening. Decreased villus swelling was detectable in one of these cases. Tongue and finger villi in the 2 remaining mild cases did not undergo further villus joining, treatment causing a decrease in swelling with increased villus tapering and mobility that resulted in normal appearing mucosa.

TABLE I

APPEARANCE OF THE JEJUNAL MUCOSA IN TROPICAL SPRUE. EFFECT OF FOLIC ACID AND VITAMIN B_{12}

	Case number	Initial biopsy	Number of post-treatment biopsy specimens	*Dissecting microscope appearance* Villus pattern * Pre-treatment	Final post-treatment	Change	Other criteria (see text)
	1	Mild	14 d, 7 wk	F,T	F,T	—	improved
	2	Mild	3 d, 10 d, 20 d, 10 wk	T,L,R	L,T,R	worse	improved
	3	Moderate	9 d, 1 yr	R,L	R,L	—	improved
Folate	4	† Moderate	3 d, 9 d, 17 d, 17 wk, 6 mo	R	R,L,T,F	improved	improved
treated	5	Moderate	7 d, 10 wk	R	R	—	improved
group	6	Marked	3 d, 6 d, 6 wk, 8 wk	R	R	—	improved
	7	Marked	2 d, 12 d, 8 mo	‡	L,T	improved	improved
	8	Mild	3 d, 10 wk	T,F	L,T	worse	same
	9	Mild	7 wk, 4 mo	F,T	F,T	—	improved
	10	Moderate	5 d, 26 d	R	R	—	improved
Vitamin B_{12}	11	Moderate	7 d, 7½ wk	‡	T,L,F	improved	improved
treated	12	† Moderate	7 d, 21 d, 14 wk	R,L	R,L,T	improved	improved
group	13	Marked	4 d, 13 d, 30 d	R	R	—	initial marked improvement, worse at 30 d
	14	§ Marked	3 d, 6 d, 33 d	R	R,L	improved	improved

* R = Convoluted ridges; L = leaf forms; T = tongue forms; F = finger forms; (predominant form is listed first.)

† Pretreatment biopsy, distal duodenum; occasional Brunner's glands present.

‡ Pretreatment biopsy, flattened cobblestone pattern composed of short, blunt villus projections, some joined to form rosettes, suggesting rapid acute development.

§ Patient received 30 μg vitamin B_{12} per day for 6 days. No further vitamin B_{12} was given. A low folate diet was started 5 days before the final biopsy was performed.

Some of the convoluted ridges seen in 2 examples with moderate changes (cases 4 and 12, Table I) were replaced by leaf, tongue or finger villi, an improvement. No change in villus pattern occurred in the other 4 cases of moderate severity. Decreased swelling and increased height and tapering of the villi were clearly evident in all cases of moderate degree.

The influence of treatment on markedly abnormal specimens was extraordinary. Short villi sprouted from one of the flattened mucosal surfaces within 2 days. In the 3 cases in which biopsy was made after 4 to 6 days of treatment, tall, swollen, and translucent, finger, tongue and leaf villi were present (Figs. 1, 2, 3 and 4). Subsequent sampling disclosed other noteworthy features: Coincident with the subsidence of villus swelling observed after 4 to 6 weeks of treatment, tissue translucency was altered by the development of epithelial transparency. Subepithelial capillaries seen through the clear epithelial covering were sharp and distinct. The capillary network in the villus cores appeared simplified, one or more straight branches terminating at the villus extremity in a slight dilatation, an aspect possibly produced by columns of red cells seen end-on. Convoluted ridges and leaves predominated in the folate treated cases after 2 and 8 months of treatment, respectively (Fig. 5). Although dramatic emergence of tongue and finger villi occurred in case 13 (Table I) after 4 days of vitamin B_{12} treatment, the tissue obtained at 30 days disclosed reversion to flattened, closely spaced convoluted ridges. Worsening of this patient's clinical condition dictated the addition of folic acid to his regimen which resulted in renewed improvement, both clinically and in the appearance of subsequent tissue samples. Figures 3 and 4 illustrate the rapid growth of villi from the flattened mucosal surface in case 14. At 33 days, blunt-tipped leaf villi predominated over tongue forms in this case. Treatment with vitamin B_{12} for 7 weeks is illustrated in Figure 6, a case of moderate severity not included in this study.

Summarizing the 14 cases, the villus pattern improved in 5 patients; 3 had moderate, 2 had marked changes in the pretreatment specimens. An increased proportion of leaves worsened the mucosal appearance in 2 cases with mild lesions. The villus pattern was unchanged in 7 cases. Improvements referred to as 'other criteria' were noted in 12 cases; 1 case with mild lesions was unchanged and 1 case with marked lesions worsened after an initial period of improvement. Restoration of normal appearing mucosa was effected in 2 cases of mild degree.

As to the time required for a detectable response to occur, cases with marked abnormality responded to a minimum of 2 to 3 days' treatment; moderate lesions were visibly improved within 9 days to 7 weeks; im-

provement was delayed for 7 to 10 weeks in cases with mild alterations.

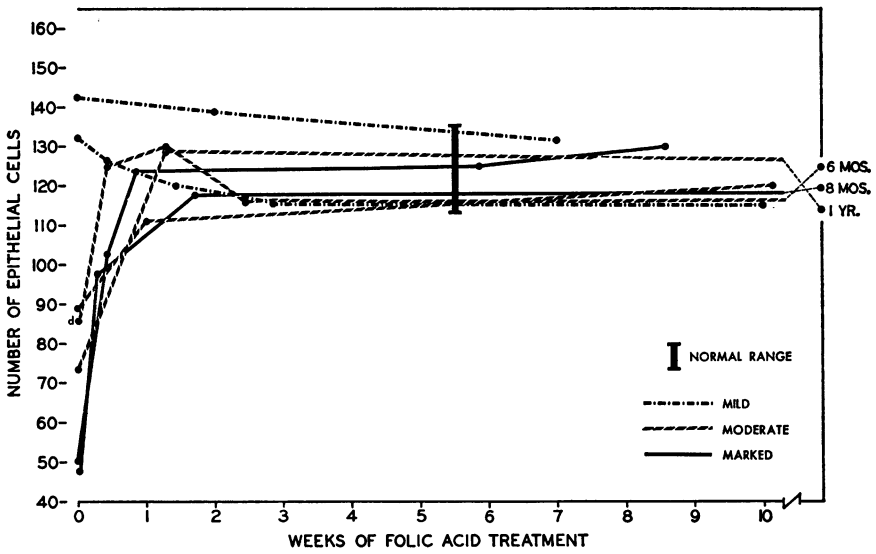
Significant differences between folate and vitamin B_{12} treatment could not be detected by dissecting microscope examination. The worsening of one marked lesion after a 30-day treatment with vitamin B_{12} brings up the possibility that at times vitamin B_{12} may be a less effective form of treatment than folic acid in the treatment of mucosal atrophy in tropical sprue.

Histologic Observations

Epithelial Population. Both folic acid and vitamin B_{12} administration elicited increases in the epithelial population, especially notable in cases with moderate and marked abnormality (Text-figs. 1 and 2). The 2 cases with mild lesions in each treatment group disclosed slight downward trends of questionable significance. After approximately 1 month, all folate treated cases fell within the normal range. The epithelial cell counts from vitamin B_{12} treated specimens were more variable with 3 curves ending below, and 1 above the normal range, but periods of follow-up were shorter than in the folate group.

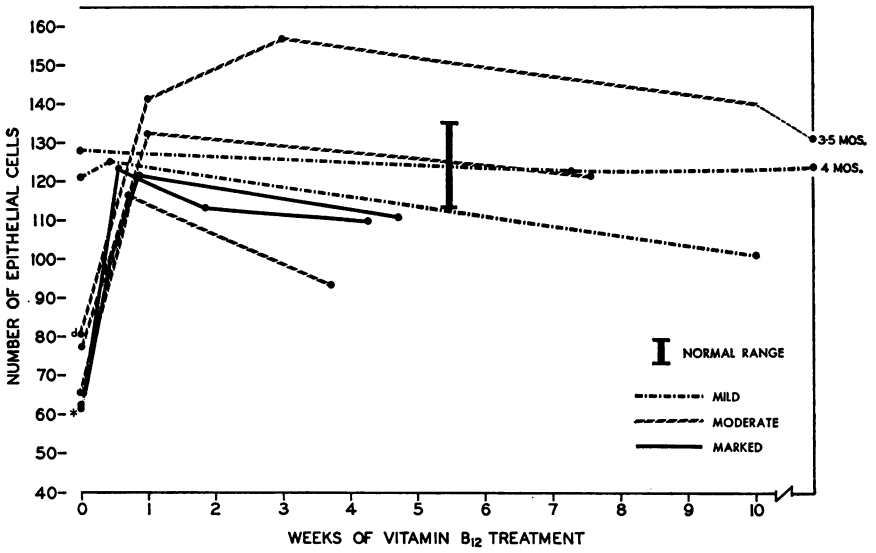
Mucosal Thickness. The increase in mucosal thickness occurring with either folic acid or vitamin B_{12} administration, was nearly complete within 2 weeks (Text-figs. 3 and 4). The thickening of atrophic or markedly abnormal specimens was principally due to the emergence of villi, as can be seen in photomicrographs depicting the response to treatment with folate (Figs. 7 and 8), or vitamin B_{12} (Figs. 10 and 11). In these cases, the largest portion of the expanded epithelial population was diverted toward covering the new villi. Meanwhile, that portion of the cell population lining the crypts showed a minimal transient rise in 2- to 4-day post-treatment tissue samples, then fell slightly in subsequent specimens, and remained relatively stationary at a mildly elevated level. In other words, the crypt cell population changed minimally while the number of villus cells increased dramatically. As a result, the villus height–crypt depth ratio improved. Thickening of the mucosa as a consequence of increased villus height also occurred in cases of moderate severity, but was not as striking as in specimens with atrophy. The 2 mild folate treated and 2 mild vitamin B_{12} treated cases were the thickest pretreatment specimens; none of these showed an increase.

Crypt Cell Nuclei. The effects of folate and vitamin B_{12} on the enlarged crypt epithelial nuclei found in this disease are summarized in Text-figs. 5 and 6. Initial reduction in nuclear size followed either form of treatment, and was most marked in cases with atrophy, less conspicuous in those of moderate degree. In no instance was normal nuclear size achieved. A tendency to rebound toward renewed nuclear enlargement



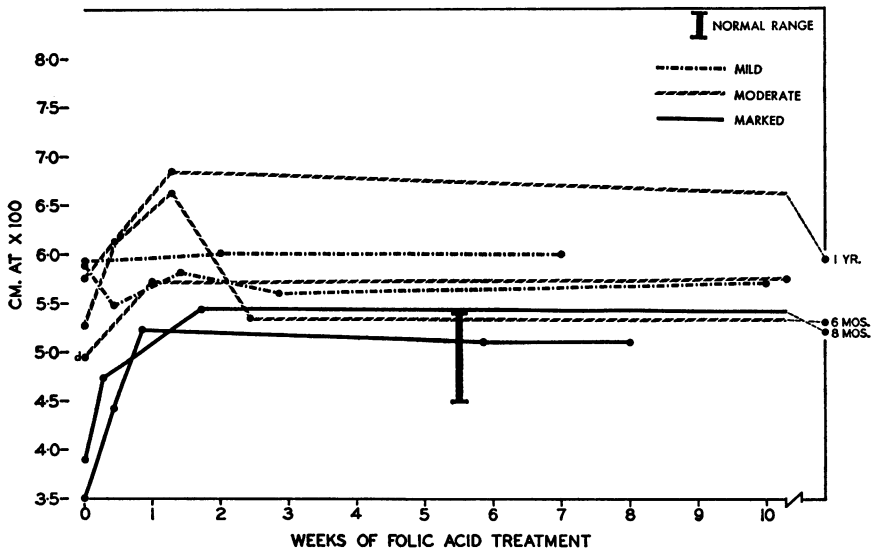
TEXT-FIG. 1. Average number of epithelial cells appearing from the middle of a crypt base to the middle of an adjacent villus tip in serial jejunal biopsy specimens during treatment with folic acid. Each curve represents a single patient. Points appearing at zero on the abscissa are pretreatment values.

In all Text-figures 'd' signifies duodenum.

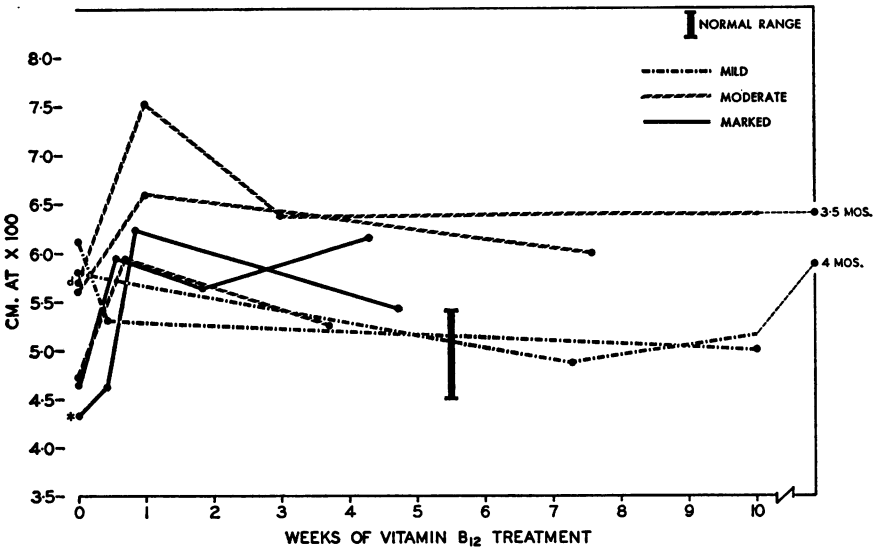


TEXT-FIG. 2. Average number of epithelial cells appearing from the middle of a crypt base to the middle of an adjacent villus tip in serial jejunal biopsy specimens during treatment with vitamin B_{12}. Each curve represents a single patient. Pretreatment values appear at zero on the abscissa.

In all Text-figures portraying data from vitamin B_{12} treated patients, the asterisk denotes a patient who received 30-μg injections of vitamin B_{12} on each of 6 consecutive days at the beginning of the study; no further medication was given. A low folate diet was started 5 days prior to the final biopsy.



TEXT-FIG. 3. * Overall mucosal thickness in cm obtained from photomicrographs during folic acid treatment. Each curve presents data from a single patient; each point is the average thickness of a single biopsy specimen.

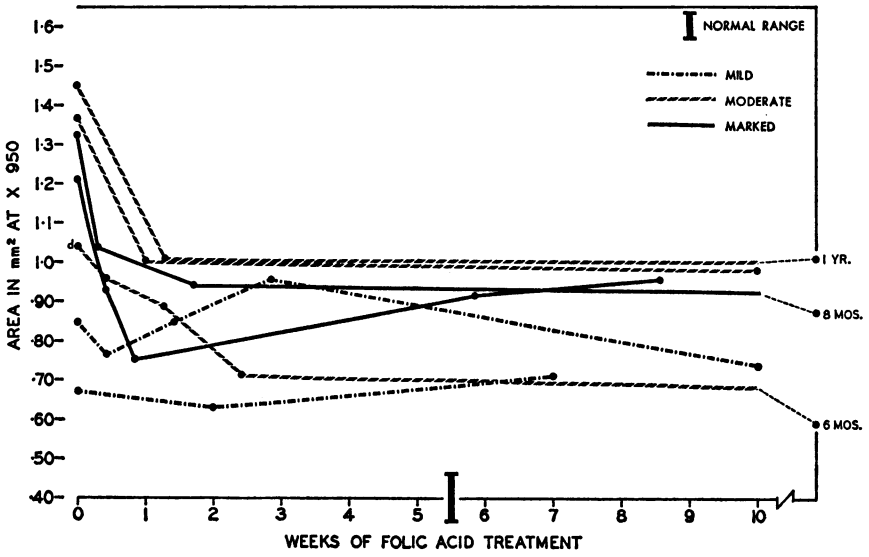


TEXT-FIG. 4. * Overall mucosal thickness in cm obtained from photomicrographs during vitamin B_{12} treatment. Data are presented as in other text-figures.

* It is possible that the normal range given for overall mucosal thickness is somewhat low. Other workers provide higher figures for normal thickness of the jejunum, viz., Shiner (696.9 $\mu \pm 128.4$) and Astaldi (576.6 $\mu \pm 73.6$). Discrepancies may be due to measurements taken from differing levels of the jejunum (thickness diminishes from proximal toward distal jejunum), or using muscularis mucosae rather than crypt bases as the lower border of measurement.

is suggested by some of the curves in the vitamin B_{12} treated group.

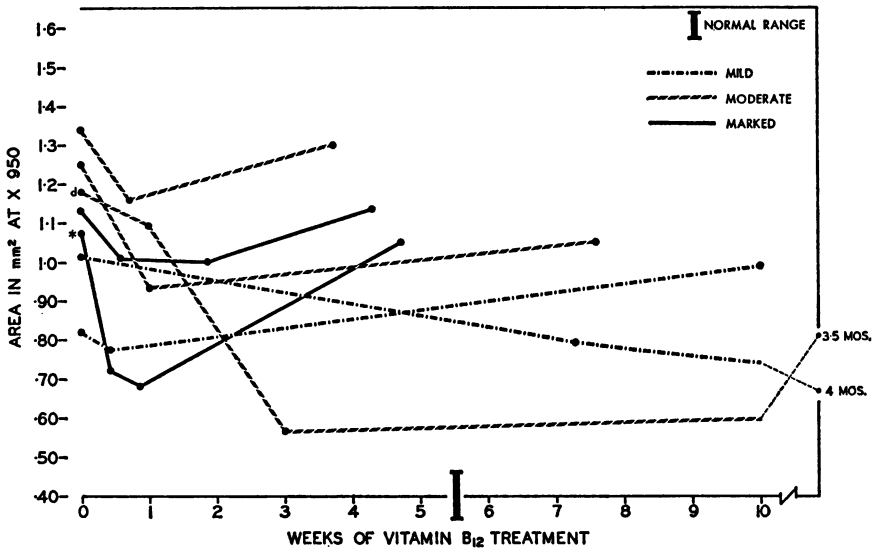
In cases with atrophy, folate treatment altered nuclear structure more effectively than vitamin B_{12}. This difference was not as apparent in moderate lesions, or not detectable in some mild ones, presumably because initial biopsy specimens showed a less striking contrast with



TEXT-FIG. 5. Area in mm² of jejunal crypt cell nuclei during folic acid treatment, derived by planimetry from camera lucida tracings. Data from a single patient are given in each curve; each point is an average derived from 50 crypt cell tracings. Pretreatment values appear at zero time.

post-treatment appearance. In cases with atrophy, however, abnormalities such as vacuolization, chromatin clumping and nucleolar enlargement, began to recede within the first 2 to 10 days of treatment. The decrease in size and subsidence of nuclear abnormalities was associated with a change in shape of a majority of the nuclei, from round to an elongated oval. At the same time, the configuration of the entire cell changed. Large cuboidal-shaped cells were supplanted by tall columnar cells with their nuclei spaced at close intervals, effecting an appearance of greater compactness of the cells. The cytoplasm became more dense and deeply stained with eosin, to enhance the impression of greater compactness. It seems probable that reduction in crypt depth may be attributed in part to this change in cell shape.

Argentaffin Cells. The increased number of argentaffin cells found in tropical sprue provided a further method of assessing the effects of folate and vitamin B_{12}. Cases of marked and moderate severity treated with folate showed a rapid, consistent diminution in the argentaffin cell pop-

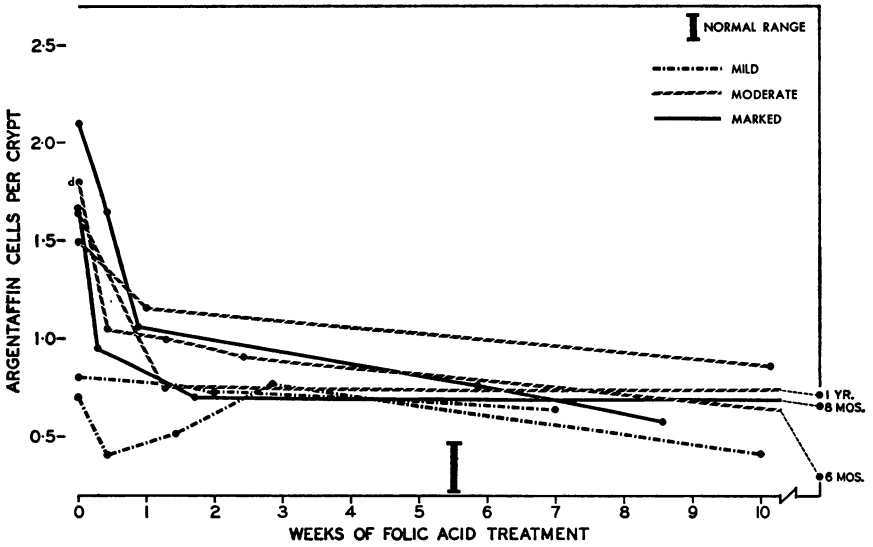


TEXT-FIG. 6. Area in mm² of jejunal crypt cell nuclei during vitamin B_{12} treatment. Data obtained and presented as in Text-figure 5.

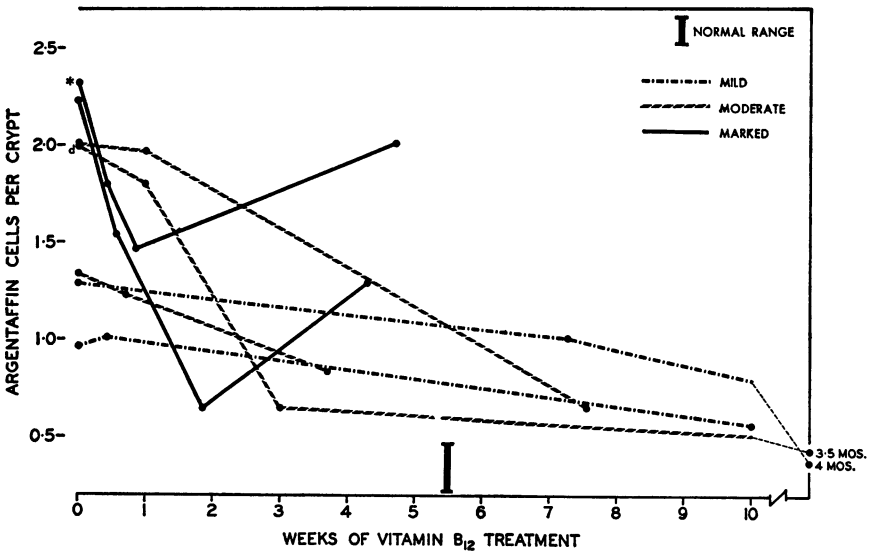
ulation (Text-fig. 7). Among the vitamin B_{12} treated cases, 2 cases with marked abnormality initially pursued a sharp, downward slope followed by an upward rebound observed in 1-month post-treatment tissues (Text-fig. 8). The numbers of argentaffin cells in the remaining vitamin B_{12} treated cases generally declined, more definitely in cases of moderate than in those of mild severity. A reduction to normal argentaffin cell frequency was achieved after 10 weeks to 6 months of treatment in 4 cases (3 mild, 1 moderate); all others continued to exhibit slightly greater than normal numbers of cells during the periods of follow-up.

Mitosis Frequency. The brisk mitotic activity precipitated by either form of treatment in cases with atrophy coincided with expansion of the epithelial population (Text-figs. 9, 1 and 2). The mitosis indexes of folate treated specimens subsided to normal or near normal. Mitoses remained increased in one case after 30 days of vitamin B_{12} treatment (Text-fig. 9). In another vitamin B_{12} treated case, a subnormal mitosis index was recorded on the 33rd day, when the patient had not received any treatment for the preceding 26 days. Although his serum level of vitamin B_{12} was normal on day 33, he had received a low folate diet for the 5 days preceding biopsy. Pertinent to the restriction of dietary folate is the report of Frazer, Fletcher, Sammons and Williams [13] of diminished mitotic activity in the intestinal crypts of rats given a folate deficient diet.

In all cases of moderate severity, subjective evaluation of mitotic activity revealed increases soon after treatment was started. This was not



TEXT-FIG. 7. Average number of argentaffin cells per crypt during folic acid treatment. Data from a single patient are presented in each curve. Points appearing at zero time are pretreatment values.

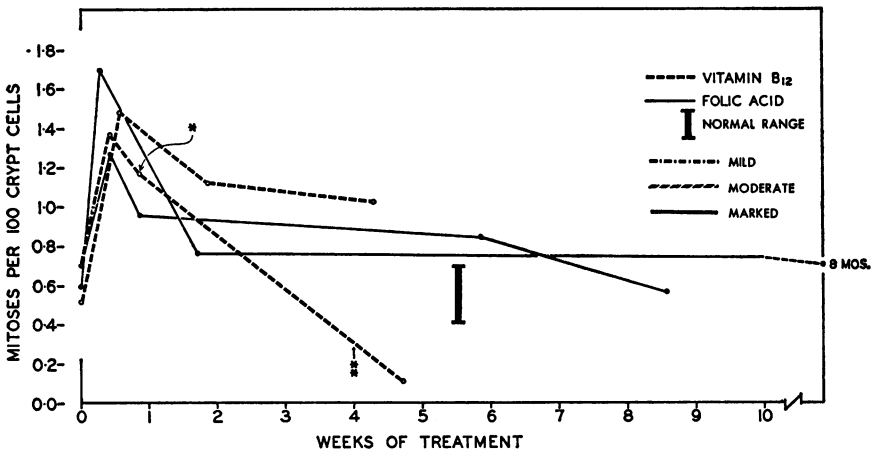


TEXT-FIG. 8. Average number of argentaffin cells per crypt during vitamin B_{12} treatment. Data are portrayed as in Text-figure 7.

observed in those with mild lesions. Later, reduction of the mitosis index was usually observed, but slight elevations in mitotic activity were frequently noted in final biopsy samples.

Additional Histologic Findings. Transient mucosal edema was apparent in 4- to 9-day post-treatment specimens in cases of atrophic and

moderate severity, but was not noted in those with mild lesions. Plasma cells continued to predominate over macrophages and monocytes, lymphocytes and eosinophils in the interstitium. During the varying periods of follow-up, density of the inflammatory infiltrate in the lamina propria remained unchanged in all cases with atrophy. In cases with moderate



TEXT-FIG. 9. Mitosis indexes of 4 atrophic jejunal biopsy specimens before and after treatment with folic acid or vitamin B_{12}. Points appearing at zero time are pretreatment values.

* Last dose of vitamin B_{12} given.
** Special diet low in folic acid started.

lesions, it was unchanged or lessened, and in all cases with mild lesions it diminished. Lymphocytic infiltration of the epithelium, which was evaluated separately, did not abate in 3 cases with atrophic mucosa but increased in 1 folate treated case with atrophy. Lymphocytes transmigrating epithelium were either unchanged in number or diminished in cases of moderate severity; 3 of 4 cases with mild lesions showed waning of the epithelial infiltrate. Two general impressions stemmed from these observations: a) the greater the severity of the initial lesion, the more likely is inflammation to persist, and b) when inflammation recedes, it does so gradually over a period of weeks or months.

Stained sections of normal jejunum viewed with low magnification disclose periodic indentations of the lumen surface of the villi producing an appearance of epithelial scalloping. The scallops are accentuated by villus contraction but are also maintained when the villus is fully extended. This normal histologic feature is gradually lost during the development of the characteristic tropical sprue lesion, and is often entirely absent in the presence of atrophy. In this study, a few epithelial scallops reappeared after 2 to 4 days of treatment in cases with atrophy initially

bereft of scalloping. Improved scalloping was detected in cases of moderate severity after 7 to 17 days of folate administration, after 7 to 26 days of vitamin B_{12} treatment. Return to virtually normal scalloping had taken place in 3 cases with mild lesions after 7, 10 and 16 weeks of treatment. Residual slight defects in epithelial scalloping were observed in all other cases throughout the period of study.

The final biopsy specimens in 2 cases with mild lesions that were restored to a normal dissecting microscope appearance exhibited histologic features of chronic nonspecific jejunitis. Although histologic improvement was noted in all final biopsy tissues in each of the remaining cases, there were significant residual abnormalities (Figs. 9 and 12). There was variability from case to case and in the extent of abnormality of each feature, but characteristically there remained: slight deepening of the crypts and consequently shortened villi, cytoplasmic vacuolization and lymphocytic infiltration of the epithelium, mild nuclear enlargement with variability in size and shape of crypt cells, mild defects in epithelial scalloping, slight increases in argentaffin cells, and interstitial inflammation. Characteristics of these treated cases which distinguished them from untreated, active or symptomatic borderline-mild cases contained in our files included: the more numerous villus epithelial scallops and the variability of nuclear shape and size of crypt cells with predominance of elongated nuclei.

Discussion

Tropical sprue patients with marked body wasting, severe anorexia and weakness often experience a sense of well-being within hours, and a ravenous appetite within a few days after onset of treatment with folic acid or vitamin B_{12}. Reversion of the bone marrow from megaloblastic to normoblastic erythropoiesis occurs within the first week of treatment with a resultant outpouring of reticulocytes into the peripheral blood. Another morphologic manifestation of this phenomenal response to treatment is a surge of epithelial proliferation in the intestine, as detailed in this study. Once the intestinal epithelial cell deficits have been corrected, however, further mucosal improvement occurs more slowly. Although body weight may be increasing, various tests agree in showing that restoration of intestinal absorptive capacity to normal is delayed.

In this study morphologic abnormalities also continued to linger. The tendency for abnormal villus patterns to persist while other features of the dissecting microscope appearance improve is important. It is well known that tropical sprue presents a broad and varied clinical spectrum, marked commonly by a succession of relapses and remissions. In Puerto Rico, we have detected malabsorption and borderline jejunal and bone

marrow changes in subjects with minimal symptoms. We have also noted spontaneous remissions in cases of mild to moderate severity. In this light, and particularly in a community where episodic diarrhea is an acceptable norm, abnormal villus patterns may be expected to develop over a period of time in a susceptible individual, perhaps intermittently and without the production of bothersome symptoms. As we have noted, abnormal villus patterns tend to persist. This may account for the prevalence of abnormalities in control subjects residing in endemic areas. Baker and associates,[14] in South India where a malabsorption syndrome is endemic, has reported abnormal villus patterns in all of 25 control subjects. Abnormal villus patterns have also been seen in some control subjects in Puerto Rico.[15] The dissecting microscope observations in this study suggested that villus height and villus swelling were particularly affected by treatment with folate and vitamin B_{12}. The less constant effect on villus patterns with their tendency to persist indicated that less reliance may be placed on these patterns. An earlier report of pretreatment biopsy specimens from tropical sprue patients in Puerto Rico[8] called attention to the common lack of correlation between the villus pattern and the degree of histologic change. Short finger-forms occasionally made up the predominant pattern in severe lesions, and sometimes mild histologic change was seen with convoluted ridges. It may be suggested that villus height and villus swelling also provide reliable criteria than villus patterns in evaluating pretreatment biopsy tissues.

The significance of persisting abnormal villus patterns is not known, but it appears unlikely that they signify permanent, irreparable damage. Ten Thije[16] has reported restoration of normal finger villi from a convoluted ridge pattern in a patient with celiac disease after exclusion of gluten from the diet for 7 months. In tropical sprue, the continuing presence of an unknown etiologic factor could explain persistent abnormal mucosal patterns. The residual histologic abnormalities after correction of vitamin deficiency, and the tendency for relapse to occur, offer support to this proposal.

Histologic abnormalities do not occur in the jejunum with simple deficiencies of folate[4,11] or vitamin B_{12}.[5,6] Consequently, malabsorption of folate with an ensuing deficiency is more likely the result, rather than the cause of the jejunal lesion in Puerto Rican tropical sprue. In a previous report,[8] total epithelial cell counts were within the normal range in mild jejunal lesions, but consistently fell below normal in moderate, severe and atrophic specimens. Based on assays of serum, deficiencies of folic acid and vitamin B_{12} also appeared more marked in the more advanced cases of tropical sprue.[17] This suggests that epithelial production may be correlated with the availability of folate or vitamin B_{12} and be

likened to erythrocyte production by the bone marrow. It is pertinent to the current study that early stages of the typical mucosal lesion precede disturbed epithelial production and low serum levels of the vitamin. The mildest jejunal lesion which should have been easily corrected if it were due to simple deficiency, was in fact the most resistant initially to folate treatment. From this it appears that vitamin deficiency *per se* is not the primary precipitating factor initiating the lesion.

Despite the apparent effectiveness of folic acid, and its administration in abundant quantities for prolonged periods, the jejunum in 6 of 7 cases studied was not restored to normal. In a similar group of patients, abnormalities remained after adequate vitamin B_{12} treatment. It is improbable that the persisting morphologic abnormalities resulted from mucosal deficiency of folate or vitamin B_{12}. Rather, the residual abnormalities may constitute a basic lesion of malabsorption upon which changes related to disordered folate-B_{12} metabolism are superimposed. The abnormalities persisting after treatment had many features in common with untreated borderline-mild lesions. It is possible that alterations seen in the early stage of tropical sprue constitute a similar basic lesion upon which may be superimposed the disturbance in folate-B_{12} metabolism that eventually results in low serum levels of the vitamins.

In tropical sprue, most nutriments are poorly absorbed. Decreased absorption of folate has been demonstrated by measurements of serum values and urinary excretion following oral administration of folic acid (pteroylglutamic acid).[18–20] Observations made in our morphologic study suggest that folate reached the mucosal cells in effective amounts and without delay following oral administration of pharmacologic quantities similar to those used in the absorptive tests.[18–20] Taking the response to parenteral vitamin B_{12} as an approximate standard, the response to folate given orally was equivalent, both in time of onset and in magnitude. Thus, at the dosage level used, any impedance to absorption of folate was overcome sufficiently to produce a seemingly maximal proliferation of epithelium and other tissue effects. The possibility exists that orally administered folate may be taken up by relatively depleted mucosal cells (despite the prior parenteral "loading" usually employed) and not be passed into the blood and urine where it has been measured.[18–20] If this occurs, then the absorptive tests would exaggerate the extent of malabsorption of synthetic folic acid.

Why the intestinal epithelial cells in many cases of tropical sprue require such large amounts of folate, and why they respond to synthetic folate (monoglutamate) but apparently fail to utilize dietary folates (polyglutamates) is unknown. Both of these questions may find a plausible explanation in functional impairment of folate-B_{12} metabolism within

the abnormal, immature appearing epithelial cells. Large amounts of vitamin B_{12} or a simple form of folate could partially restore their proper utilization and function by loading the system, and have salubrious morphologic effects on the tissue. The basic underlying defect in folate-B_{12} metabolism would remain, however, accounting for the failure of treatment to restore the mucosa to morphologic normality, and accounting for the frequency with which relapse occurs when treatment is withdrawn. If this hypothesis is correct, one may expect a condition of maximally defective utilization to develop, i.e., the mucosa would no longer respond to vitamin saturation. This saturation may prevail in those patients with chronic diseases who develop clinical and laboratory evidence of relapse while under treatment with large doses of folic acid.

Summary

The purpose of this study was to report the tissue responses of the upper small intestinal mucosa of tropical sprue patients to treatment. Jejunal biopsy specimens were obtained from 14 patients with this disease in Puerto Rico before and after treatment with large doses of either folic acid, given orally (7 patients), or vitamin B_{12} given intramuscularly (7 patients). Pretreatment specimens showed mild (4), moderate (6), or marked (4) changes. Folic acid or vitamin B_{12} were given throughout the study, during which multiple additional biopsy samples were obtained. Final biopsies were made after 26 days to 1 year of treatment. Pre- and post-treatment specimens were compared using the dissecting and compound microscopes; histologic changes were quantitatively measured.

Following treatment with either folate or vitamin B_{12}, markedly abnormal specimens exhibited a rapid, profound response; the effect on moderately abnormal specimens was prompt and definite, while mildly abnormal specimens exhibited delayed, minimal change. Villus projections reappeared and a surge of mitotic activity restored the epithelial population in specimens with atrophy. Nuclear abnormalities such as enlargement, chromation clumping, vacuolization and prominent eosinophilic nucleoli receded to a considerable extent. The transformation in size and shape of epithelial cells and their nuclei as they ascended the villi became more normal. Argentaffin cells became less numerous. Residual abnormalities remained in 12 of 14 cases, however, and frequently appeared stationary despite prolonged and abundant therapy. Convoluted ridges and leaf-shaped villi tended to persist. Histologically, there were slight depressions of the villus height–crypt depth ratios, mild epithelial abnormalities, small increases in numbers of mitoses and argentaffin cells, and inflammation.

In cases of moderate or marked severity, the effects of folate given orally were equivalent, both in time of onset and in magnitude, to those of vitamin B_{12} given by injection, which indicated that when given in large doses synthetic folate is taken up by the cells in effective amounts and without delay. The initial resistance of cases with mild lesions to treatment and the minimal histologic response, supported the hypothesis that the jejunal lesion is not initiated by vitamin deficiency *per se*. Failure of vitamin treatment to restore the mucosa to normal suggested that there is a basic lesion in tropical sprue upon which changes related to disordered folate-B_{12} metabolism are superimposed. Following correction of the vitamin deficiencies, the residual abnormalities which we observed may indicate that the mucosa is still affected by a primary inciting cause, or, that there is a mucosal defect in folate-B_{12} metabolism which persists, or both.

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LEGENDS FOR FIGURES

FIG. 1. Case 6. Pretreatment jejunal biopsy specimen with flattened convoluted ridges, suggestive block formation and atrophic thinning of the mucosa. Minute pits lined up in a sulcus between two ridges are visible at the lower left and elsewhere in the photograph. The pits are thought to be crypt orifices; they become apparent when all excess fluid is drained off. × 36.

FIG. 2. Biopsy specimen from jejunum illustrated in Figure 1 after 6 days of folic acid treatment (15 mg per day). Finger, tongue and leaf villi are edematous and translucent. Dilute hematoxylin was used to create the shading that emphasizes the height of the villi. × 36.

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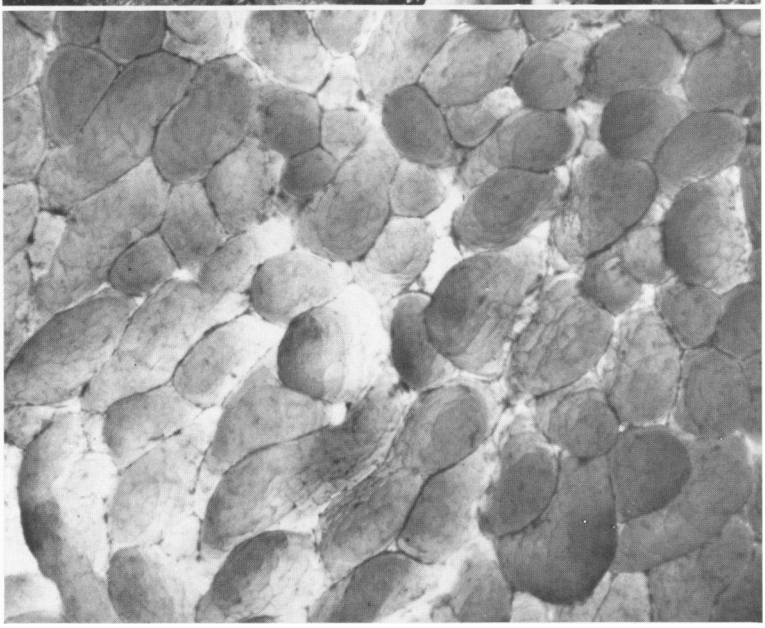


FIG. 3. Case 14. Pretreatment jejunal biopsy specimen with flattened convoluted ridges, block formation and microscopic evidence of atrophy. Pits are also visible in the sulci. × 36.

FIG. 4. Biopsy specimen from same jejunum shown in Figure 3 after 6 days of vitamin B_{12} treatment (30 μg per day). Swollen finger, tongue and leaf villi are present. Wrinkles on the sides of the villi at the upper left are made more distinct by the application of dilute hematoxylin and are not due to drying. They are thought to coincide with epithelial scalloping seen histologically. × 36.

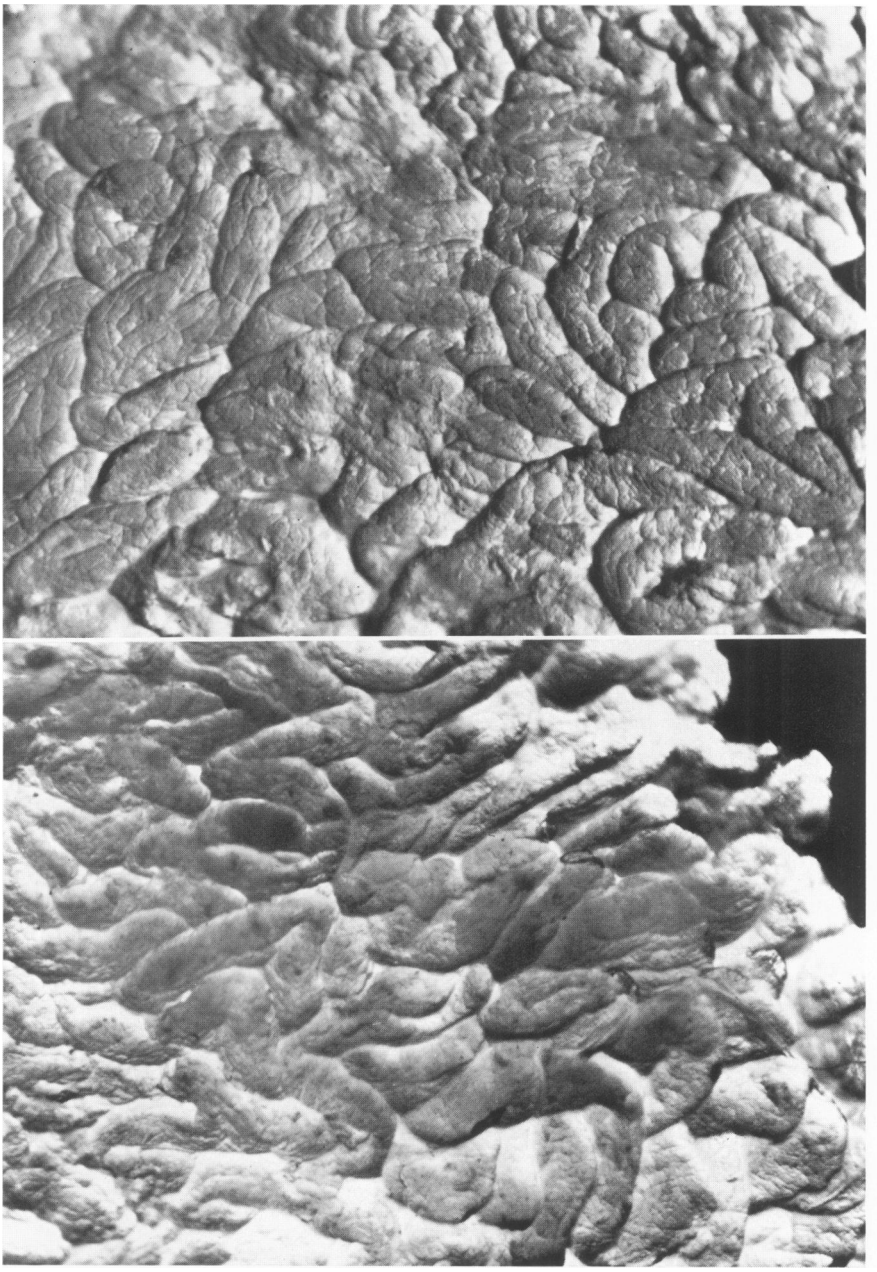


FIG. 5. Biopsy specimen from jejunum illustrated in Figures 1 and 2 after 2 months of folate treatment (15 mg per day). Convoluted ridges, closely spaced and with rounded crests, are present. Mobility of the ridges permits insertion of a fine glass probe into the moderately deep intervillus spaces or sulci. Wrinkling of the exposed villus crests is due to slight dryness. × 36.

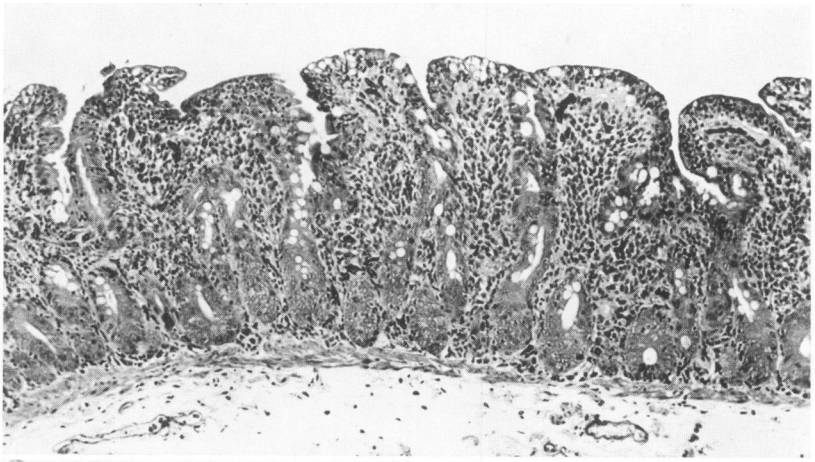
FIG. 6. Jejunal biopsy specimen after 7 weeks of vitamin B_{12} treatment (30 μg per day for 1 week followed by 30 μg per week for 6 weeks). Convoluted ridges and leaves are present. There is tapering of some of the villus tips. Villi that are out of focus are taller than their neighbors. The pretreatment specimen had moderate villus abnormalities with predominant convoluted ridges. × 36.

All photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 7. Pretreatment jejunal biopsy specimen in case 6 showing atrophy, marked lengthening of the crypts, blunting of the villi and inflammation. Individual epithelial cells are short, tending toward a cuboidal shape. The dissecting microscope appearance of this specimen is illustrated in Figure 1. × 96.

FIG. 8. Biopsy specimen from jejunum shown in Figure 7 after 6 days of folic acid treatment. Increased mucosal thickness is apparent. Villus height has increased while crypt depth has been reduced. Epithelial scalloping has returned and the individual cells are taller. Inflammation appears to be reduced, but dispersion of cells by edema probably contributes to attenuation of the infiltrate. Dissecting microscope appearance is illustrated in Figure 2. × 96.

FIG. 9. Biopsy specimen from same jejunum shown in Figures 7 and 8 after 2 months of folate treatment. Residual abnormalities seen at this magnification include crypt lengthening, villus shortening, suppressed epithelial scalloping and inflammation. Dissecting microscope appearance is seen in Figure 5. × 96.



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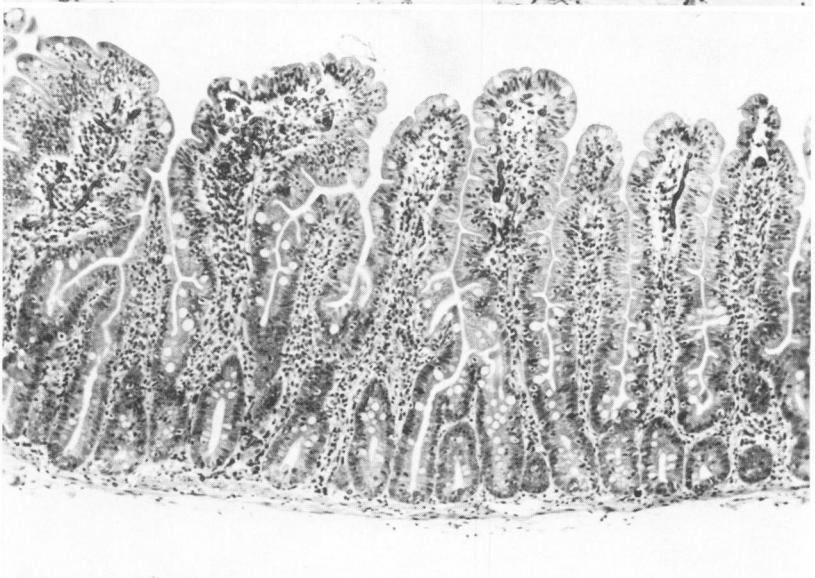

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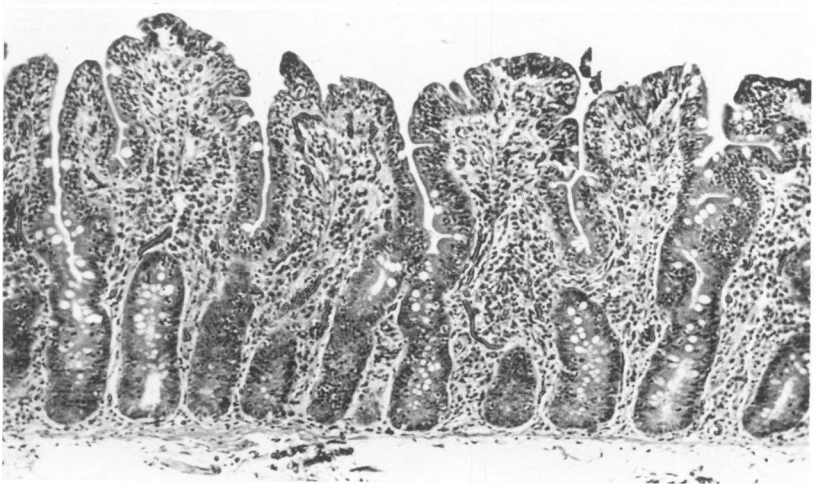

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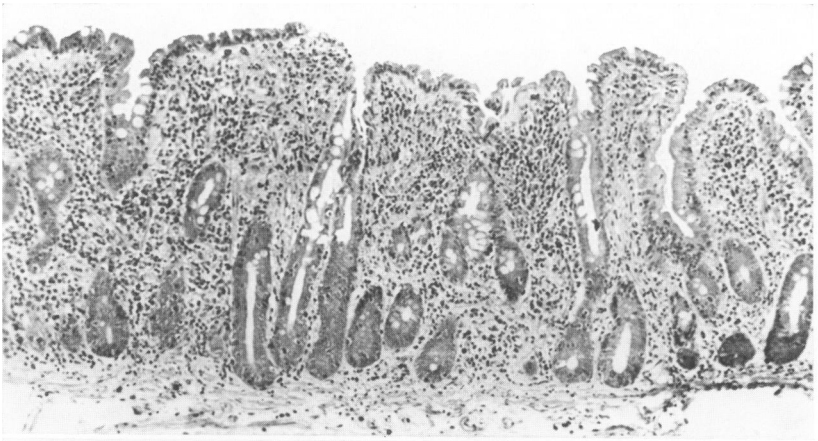
FIG. 10. Pretreatment jejunal biopsy specimen in case 14. Histologic changes are similar to those in Figure 7, although atrophic mucosal thinning is not as marked. Dissecting microscope appearance is illustrated in Figure 3. × 96.

FIG. 11. Biopsy from same jejunum shown in Figure 10 after 6 days of vitamin B_{12} treatment. Morphologic changes effected by vitamin B_{12} cannot be distinguished from those portrayed in the folate treated case seen in Figure 8. Figure 4 illustrates the dissecting microscope appearance of this specimen. × 96.

FIG. 12. Jejunal biopsy specimen after 7 weeks of vitamin B_{12} treatment. The variability of the villus pattern (illustrated in Fig. 6) corresponds to the variability of the histologic section. Tall, tapered villi associated with slightly lengthened crypts at the right of the photograph contrast with the blunt, broad villi with deeper crypts at the left. Epithelial scalloping is mildly defective and inflammation is persistent. × 96.



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