

STUDY REPORTS

1. Title: Enzyme Histochemical Studies of the Non-Specific Jejunal Lesion in Thai People

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Objective:

To evaluate: 1. The enzymatic staining activity of the mucosa of the small intestine of the Thai people. This would show whether the mucosa with "mild jejunitis" or non specific abnormalities displays any enzymatic aberration. It would also help establish a base line for further comparison with the staining pattern seen in other enteric diseases.

2. Enzymatic pattern in normal subjects after manipulation of the intestinal tracts with some drugs like antibiotics.

3. Enzymatic staining pattern in various enteric conditions.

Description:

Biopsies were obtained from the mucosa of the proximal jejunum with the Crosby-Kugler biopsy capsule. Subjects were fed a high fat diet by the addition of 75 gm of butter per day. All subjects fasted for 10-14 hours before biopsy. The tissue was divided and one piece was fixed in 10% buffered neutral formalin for gross and microscopic examination. The other piece was oriented villus side up on a block of fixed liver or kidney tissue and quick frozen sectioning at -20°C . Eight micron sections were picked up on cover slips, air dried, and stained on the same day. In a few cases sections were stored in an air-tight container at -70°C and stained on the following day. Tissue was examined for the activities of alkaline phosphatase, acid phosphatase, non specific esterase, succinate dehydrogenase, DPN diaphorase, and glucose-6-phosphate dehydrogenase. Unstained cryostat sections were fixed in formalin, and stained with oil-red O and hematoxylin. Controls for the enzymatic staining were based on the following procedures: 1. Simultaneous incubation of fresh frozen sections of mouse liver and intestine with the sections of human intestine. 2. Incubation of sections in media without specific substrate. 3. Simultaneous incubation of sections from different cases. 4. The use of specific inhibitors as described for the various enzymes. The activities of the enzymes were graded roughly 0 to 4+, according to the overall intensity of staining.

Progress:

One hundred and four biopsies obtained from three groups of patients were studied:

1. Thirty-nine biopsies from twenty-eight Thai adults with normal intestinal absorption of xylose and fat.
2. Sixty biopsies from the above subjects receiving neomycin and biopsied before, during and after the drug.
3. Five biopsies from one Thai patient with tropical sprue.

Results:

Enzymatic staining activities.

Group I. All subjects in this group showed histological evidence of "Nonspecific jejunal abnormality". The enzymatic staining reactions were similar to the pattern seen in the mucosa of normal North American controls. Alkaline phosphatase stained strongly in the brush border of the absorptive columnar epithelial cells of the villi, ending abruptly at the level of the crypt. Activity was also observed in occasional blood vessels in the lamina propria. The activity of ATPase was strong in the brush border of the absorptive cells of the villi and was not present in the crypt. A small amount of reaction product was observed in the apical part of the cytoplasm of absorptive cells and also in occasional vessels in the lamina propria and muscularis mucosa. Acid phosphatase activity was localized to the supranuclear part of the absorptive epithelial cells, as well as in macrophages within the lamina propria. Faint reaction was observed in crypt cells. Strong activity was noted in Paneth cells at the bottom of the crypt. The activities of succinate dehydrogenase, glucose-6-phosphate dehydrogenase and DPN diaphorase were prominent in the peripheral part of the cytoplasm of the absorptive epithelial cells of the villi, particularly in the apical and supranuclear portion of the cells. Activity in the crypt was much less intense. Esterase activity was localized mainly to the supranuclear portion of absorptive epithelial cells. Lesser activity was noted in crypt cells. Esterase activity was also observed in occasional macrophages.

The histochemical staining pattern was consistent in slender finger villi. Occasionally patchy areas of minimally decreased activity (3+) for ATPase, succinic and G-6-P dehydrogenase were observed in short and blunted villi. This might represent sectioning along the long axis of a leaf or ridge shaped villus. Acid phosphatase granules in the supranuclear portion of the absorptive cells in Thai intestinal mucosa appeared to be more abundant than in comparable specimens from American subjects studied in our laboratory. Acid phosphatase activity in the lamina propria was also more prominent in the Thai specimens because of the presence of greater numbers of macrophages.

Group II. Fourteen subjects received neomycin orally (2-8 gm/day). In ten subjects neomycin was given for 7 days and in 2 subjects for 3 days. In two subjects a single 2 gm dose of neomycin was given orally, and biopsy performed 6 hours later and again at 28 hours. All patients showed biochemical evidence of malabsorption, including the two cases given a single dose of neomycin. These abnormalities returned to normal over several days when the drug was stopped. In four out of subjects receiving a 7 day course of neomycin, 4 gm/day, patchy areas of decreased staining (2-3+) in the activities of ATPase, alkaline phosphatase, and succinic dehydrogenase. This was accompanied by a slight increase in the activity of acid phosphatase in the macrophages and in occasional absorptive epithelial cells. In one subject given 8 gm of neomycin daily, slight decrease (3+) in the activities for ATPase, alkaline phosphatase, and succinic dehydrogenase was noted after 3 days of drug administration, while at 7 days activity was markedly decreased (1+ to 2+). Acid phosphatase and esterase activities in macrophages and occasional absorptive epithelial cells were slightly increased. In two cases given a single dose of drug, a decrease in the staining activities of all enzymes at 6 hours was observed. Twenty-eight hours after neomycin the staining activity in both cases returned to normal. In two cases, one biopsied at 5½ hours, and the other at 24 hours after 2 gm of neomycin, slight irregularity in staining activity of ATPase was observed while succinic dehydrogenase activity was markedly reduced. The activity of acid phosphatase was stronger than usual in

one case while in the other it was within normal limits. At 3 days of drug administration, the succinic dehydrogenase activity remains weak. The enzyme staining pattern returned to normal 24 hours after the drug administration was discontinued.

Group III. One Thai patient with the clinical and biochemical diagnosis of tropical sprue was studied serially. Histologic features of the jejunal biopsy revealed marked villus shortening, epithelial atypism and a plasma cell infiltrate in the lamina propria consistent with the diagnosis of tropical sprue. There was a marked decrease in the area of staining of all enzymes secondary to the loss of villus surface. The intensity of staining for succinic dehydrogenase was markedly reduced (O-1+) while ATPase was slightly decreased (2-3+) in places. The intensity of staining for acid phosphatase activity was strong in macrophages and the absorptive epithelial cells on the surface of the flattened villi. One month after initiation of folic acid therapy there was definite increase in the area of staining for ATPase and alkaline phosphatase as regeneration of villi occurred. Succinic dehydrogenase activity did not improve until two months after treatment. By three months the enzyme staining was within normal limits for Thai subjects, while the villi were still relatively short. At four months the biopsy was normal.

Lipid staining.

After 12-14 hours of fasting, 10 out of 12 biopsies from subjects in group I eating the usual low fat Thai diet, showed no visible oil red O positive droplets either in epithelial cells or in the lamina propria. The remaining 2 biopsies showed occasional but rare droplets in macrophages in the lamina propria. In contrast normal North American subjects on a high fat diet ordinarily have fat droplets in the lamina propria. Therefore our subjects were given a high fat diet on the day prior to biopsy. Under these conditions the results were comparable to normal American subjects. In group II, four patients with neomycin induced steatorrhea had fat droplets within the absorptive epithelial cells at the tip of the villi, as well as in the upper part of the lamina propria. (When one of these subjects was biopsied without fat supplementation in the diet, no fat was visible in the specimen). The patient with tropical sprue had small and large fat droplets in both the apical and subnuclear portion of the cytoplasm and in the region of the basement membrane.

Discussion:

Jejunal abnormalities in "normal" Thai small intestinal mucosa were first described by Sprinz et al. They found evidences of malabsorption based on a 5 gm D-xylose absorption test and serum carotene level and suggested that the morphological findings were compatible with early sprue. A recent study by Troncale and associates, however, showed that low income, asymptomatic Thai subjects with non-specific abnormalities in the small bowel had normal xylose excretion with the 25 gm test dose, normal vitamin B¹² and vitamin A absorption and normal fecal fat content on a high fat diet. They concluded that the jejunal lesion could not be considered an early sprue lesion. The group of patients reported here, from the same population studied by Troncale et al, showed normal enzyme histochemical staining patterns and supports this conclusion.

Two factors appear to be critical in absorption or transport through the intestinal wall, the total surface area available for absorption and the functional efficiency and integrity of the individual absorptive unit. It has been pointed out that the total surface area as well as the number of absorptive epithelial cells would be significantly decreased as the villus shape changes from finger to leaf and to convolutions. Creamer has concluded that the leaf villus has 50% of the surface area of the finger villus while a convoluted surface is reduced to 25%. Histochemical staining by distinguishing mature epithelial cells from young crypt cells may assist in the evaluation of the functional integrity of these cells. Histochemical staining also clearly reveals the separation between crypt and villus and thus directly displays the absorptive surface.

In the present study neomycin, known to cause malabsorption in normal subjects, was used to study the response of the Thai with non specific jejunal abnormalities. Changes in the histochemical staining pattern

correlated with histologic evidence of cellular injury. These included cellular atypism and increased numbers of mitotic figures and goblet cells. Because the histochemical techniques used are qualitative, actual correlation of enzyme activity with the dose of neomycin was not possible. Kent, et al have shown acute changes in enzyme histochemical staining of the small intestine from staphylococcal enterotoxin in monkeys with recovery in 24 hours. Similar acute changes have not been previously described in man. In the present study alterations in enzyme staining occurred after a single dose of neomycin. What is of greater interest is that some degree of histochemical and histologic recovery following acute neomycin injury occurred even when administration of the drug was continued. Function, however, did not improve. Although actual enzyme activity may still have been considerably depressed from control in this situation and not revealed by the staining techniques, some adaptation appeared to take place. The nature of this is not known. The lack of correlation between cellular injury as revealed by light microscopy or histochemical study and the clinical evaluation of intestinal function in tropical sprue and in the neomycin lesion may be a result of the insensitivity of the clinical methods in use.

It is generally accepted that the morphologic response of the small intestinal mucosa to injury is somewhat limited and nonspecific, regardless of the type or nature of the stimulus. Enzyme and lipid histochemical staining adds another dimension to morphologic observations. However, many problems remain to be clarified. What is the meaning of differences in degree of enzymatic staining? Why is there such poor correlation between the pattern of enzymatic staining or morphology and intestinal function? Little information is available on sequential changes in both enzyme and lipid staining in physiological as well as disease states in man. These changes may occur in hours rather than days. Furthermore, the injury due to neomycin is quickly repaired, whereas the sprue lesion is not. The effects of chronicity of the injurious factor remain to be elucidated.

Summary:

Thai subjects with non-specific jejunal abnormalities have normal mucosal enzyme activity and pattern of lipid distribution.

Administration of neomycin results in decreased activity of ATPase and succinic dehydrogenase. Lipid droplets accumulate within epithelial cells in neomycin induced steatorrhea. Histochemical staining correlates well with visible epithelial cell damage but not with absorptive function.