Study on the Pathology of Metagonimiasis in Experimentally Infected Cat Intestine*

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INTRODUCTION

Endemic foci of Metagonimus yokogawai infection have been reported in the southern part of Korea (Kang et al., 1964; Yeo et Seo, 1971; Loh et Kang, 1971; Soh et al., 1976; Chai et al., 1977; Soh et Ahn, 1978). The prevalence rate in the inhabitants was reported ranging 30-60 per cent estimated on the basis of egg detection method. Since the prevalence rate is high in the endemic areas, attentions tend to be paid to this intestinal fluke. Currently the medical importance of heterophyidiasis seems to be placed on its ectopic parasitism which could occur in the heart, brain or spinal cord (Africa et al., 1935, 1937, 1948; Ito, 1964), not only because of high mortality but also of fairly wide distribution of this fluke infection. However, in order to understand rather precisely the ectopic lesions, host-parasite relationship, pathology and immunology are very important aspects since these lesions are derived via the normal ecologic niche of small intestine.

Few papers of well-described pathology of the intestinal lesion have been available for this parasitism. Taki (1936) and Koga (1938) described the lesion as “severe catarrhal inflammation associated with wound of villous epithelial cells, mucous degeneration of villi and a marked expansion of capillaries” in the heavily infected dogs and cats. In the small intestine of rats infected experimentally with M. yokogawai, Chai (1979) observed villous atrophy with blunting of its tips, severe inflammatory reaction and vascular congestion of the stroma, and slight increase of goblet cells. Considering the relatively short duration of symptoms in experimentally (Koga, 1938) and naturally (Ito, 1964) infected human cases, these findings presumably correspond to those of acute phase of infection.

Diarrhea and/or abdominal pain has been known to be the most frequent clinical complaints among human cases. However, as for the pathogenetic explanation of these symptoms, few papers are contributory. Chai (1979) suggested the possibilities of pressure atrophy by increased amount of gas and mucus in intestinal lumen, followed by primary mechanical stimulation of the worm itself. In some parasitic disorders, such as Strongyloides stercoralis (Da Costa, 1971) or Nippostrongylus brasiliensis (Ferguson et al., 1980), the turn-over rate of small-intestinal epithelium has been known to be markedly increased which is frequently observed in malabsorption syndromes. However, the fact that whether the kinetic changes of
epithelial cells are indeed the cause or rather the consequence of the parasitic infections remains to be clarified.

In this study, as an attempt to understand the basic pathology of metagonimiasis in the normal host, morphological changes of small-intestinal mucosa as well as kinetic changes of epithelial cells were observed by different time interval in cats that were experimentally infected by giving various numbers of metacercariae of *M. yokogawai*. Along with light microscopic observations, ultrastructural changes of the epithelial cells of small intestine were also examined.

**MATERIALS AND METHODS**

1. **Collection of metacercariae and infection to cat**

Metacercariae of *M. yokogawai* were obtained from naturally infected fish (*Plecoglossus altivelis*) which had been caught from an endemic area of this infection, Tamjin river basin in Jangheung-gun, Cholla Nam Do (Chai et al., 1977). After peptic digestion, sedimented metacercariae were counted under dissecting microscope.

As a preliminary observation, a total of 12 adult cats, weighing 2.5–3.0 kg were blindly treated with pyrantel pamoate 25 mg and niclosamide 250 mg to eradicate the possible intestinal helminthic infections. Three weeks after the treatment, a stool examination by formalin ether technique was undertaken to confirm the absence of remaining helminthic or protozoal infections. There were three cats that were found to be useful for the experiment, and these cats were challenged with 80,000 metacercariae through the polyethylene capillary tube into stomach. By one week interval, the cats were killed. Gross features of small intestine were observed and subjected in this study only for gross description.

After the preliminary experiment, 72 adult cats of either sex were purchased. To eradicate possible parasitic infections they were treated exactly in the same way as in the preliminary experiment with pyrantel pamoate and niclosamide. One month after the treatment, a total of 18 cats out of 72 was found useful for this study. They were divided into three groups; control group, 10,000 and 50,000 metacercariae infection groups. Hereafter, 10,000 metacercariae infection group would be called as "light-infection group" and 50,000 metacercariae as "heavy-infection group". The number of cats in each schedule was presented in Table 1.

2. **Gross observation and preparation for light microscopy**

Cats were killed under the pentothal anesthesia. Opening the peritoneum, serosal surface was observed at first. Then, the whole small intestine was immediately removed from gastric pylorus to ileo-cecal junction. It was opened along the mesenteric border. Gross observations of intestinal contents and of mucosal changes were made. The whole length of small intestine was artificially partitioned according to Davison’s method (1903); the first 15 cm from ampulla of Vater for duodenum, next 25 cm for jejunum, proximal half of the rest for proximal ileum, and distal half of the rest for distal ileum.

After obtaining the samples for electron microscopy, the remaining whole intestine was fixed on wood plate in flat and fixed in 10% neutral formalin solution for one day. Two sections along the long axis of intestine were taken from the same part throughout the whole length of small intestine for paraffin section. Thus, two whole-length sectioned strips of small intestine were made. Hematoxylin-eosin and PAS stains were performed. More than 10 well preserved villi were selected from the mid-
portion of each part of small intestine and measured crypt length and villous height by AO micrometer.

The sectioned worms in intervillous spaces were counted in the all slides of two whole-length sectioned strips of small intestine. The whole number of worms detected throughout the small intestine was referred conventionally to the number of whole infected worms in tissue phase. The worms found floating in the intestinal lumen were not counted. Distribution of worms in each part of the small intestine was also observed. In order to observe the parasitic distribution in unit length of each part, density rate was figured out by proportional number of worms supposing that total count of sectioned worms in the whole two-strip slides be 1,000, since the actual density was very light. It was calculated as follows:

\[
\text{Density rate/1,000/cm} = \frac{\text{No. of worms detected in each part length of each part (cm)}}{1,000} \times \frac{1,000}{\text{whole No. of worms detected in small intestine}}
\]

3. Sampling and preparation for electron microscopy

One cm long section was taken at the midportion of jejunum. They were fixed in 2.5% phosphate-buffered glutaraldehyde solution at 4°C. After post-fixation in 2% osmium tetroxide solution, the sections were processed by conventional method and stained doubly with uranyl acetate and lead citrate. Microvillous findings were examined with the Hitachi electron microscope.

Scanning electron microscopy was performed only for the cats that were killed 4 weeks after infection. One cm long section was obtained from the mid portion of each part of small intestine and immediately washed with 4°C, normal physiologic saline solution. Further processes were as follows; first fixation in 2.5% phosphate-buffered glutaraldehyde solution for 40 minutes, freeze-drying for 72 hours, and double coating with carbon and gold-platinum. The findings were observed with the Akashi scanning electron microscope.

RESULTS

1. Gross pathological findings

Prominent and consistent changes were marked enlargement of mesenteric lymph nodes especially in the mesentery near the terminal ileum, and severe lymphoid hyperplasia in Peyer's patches and solitary lymphoid follicles on mucosal surface. These lesions remained somewhat unchanged with infection period or infected doses of metacercariae.

Intestinal content was greenish, foamy and watery in the preliminary study group. Greenish watery feces were also observed in the small intestine of both light- and heavy-infection groups during the first 10 days of infection. Thereafter, in both groups greenish hue disappeared and intestinal contents became less fluidy gradually as the infection time extended, even though they remained persistently mushy until the end of experiment. Neither petechial hemorrhage nor mucosal ulceration was seen in all the cats examined. Individual and chronological gross changes were described as follows.

1. Preliminary group: During the first week of infection, all cats looked sick and became physically inactive. They did not eat well. The stool was mushy and watery by the end of the first week. In the second week of infection, cats became more and more inactive, shed watery stool and refused food. Hairs became brittle. A cat of 3 weeks infection was severely dehydrated presumably due to intractable watery diarrhea and poor intake.

Serosal surface of the gastro-intestinal tract
was dry and listless representing dehydration, and this dehydration sign became more prominent with the lapse of infection time. In all of animals, serosal congestion was seen throughout the whole length of the small intestine. Mesenteric lymph nodes were generally enlarged especially in mesentery near the terminal ileum, measuring up to 1.0 cm in maximum diameter. Luminal distension was not conspicuous (Fig. 4).

During first two weeks, intestinal content was greenish foamy and liquid. In a cat after 3 weeks of infection, bulky, tarry and watery feces with fine air bubbles were seen throughout the gastro-intestinal tract including stomach (Fig. 7).

Mucosal surface appeared edematous and flat, and there was marked obliteration of transverse folds. When the intestinal contents were carefully washed out, the mucosal surface was granular showing loss of its normal velvety appearance (Fig. 6). The severity of these changes was not considerably different between each part of small intestine, although those of the jejunum seemed slightly more prominent. Peyer’s patches were markedly enlarged and solitary lymphoid follicles were also prominent in all cats.

Those gross findings remained more or less to be unchanged so far, but, in general, were deemed to become worse as the duration of infection became longer. Petechial hemorrhage was particularly looked for in the mucosal surface, but was never found in any of the cases.

2. Heavy-infection (50,000 metacercariae) group: In the 4th to 5th day of infection, all cats became somewhat sick and inactive, and loss of appetite developed. The stool became mushy and fluidly. As the infection continued, the cats ate poorly and developed persistent diarrhea associated with emaciation and loss of hairy gloss. After 3-4 weeks of infection, watery nature of the stool became gradually subsided, but not completely normalized.

In the 5th and 10th day of infection, serosal surface was somewhat dry and moderately congested. Mesenteric lymph nodes were swollen, measuring 1 cm in maximum diameter. Small intestine contained greenish and watery mucoid feces. Mucosal surface was flat and coarsely granular, exhibiting marked effacement of velvety appearance and moderate obscuring of transverse folds. There was marked enlargement of Peyer’s patches and solitary lymphoid follicles.

In the 15th day of infection, serosal dryness continued, and enlargement of mesenteric lymph nodes as well. Intestinal content was mushy rather than watery. Transverse folds were moderately obscured and villous pattern was more or less effaced. The features of mucosal lymphoid tissues were similar to those of 5–10 days infection.

In the cats of the 4th and 8th week of infection, the enlargement of mesenteric lymph nodes was similar to that of early phase of infection. However, serosal surface became rather smooth and glistening, apparently returning to the normal appearance. Intestinal content was still mushy, but mucosal surface rehabilitated its normal fine velvety appearance with restoration of transverse folds. In contrast to these normalizing tendency, Peyer’s patches and solitary lymphoid follicles were persistently enlarged.

Neither ulceration nor petechial hemorrhage was found on the mucosal surface in all cats of this group.

3. Light-infection (10,000 metacercariae) group: During 4-8 days of infection, all cats ate poorly, defecated soft or mushy stool and became slightly inactive. But the severity of these symptoms was rather mild as compared with that of preliminary or heavy-infection
group. After the 3rd week of infection, it was difficult to differentiate from the control group, except for loose and soft stool, mild emaciation, and loss of hairy gloss.

In the 5th day of infection, serosal surface was dry and slightly congestive, and there was moderate enlargement of mesenteric lymph nodes. The lumen of small intestine contained bulky and mushy feces. Mucosal surface was edematous, showing moderate obliteration of transverse folds. Peyer's patches and solitary lymphoid follicles were moderately enlarged.

In the 10th day of infection, the general gross alterations of the small intestine became more severe than those of 5 days infected cat. The findings were comparable to those of the early phase of heavy-infection group in many respects. Serosal surface was rather congestive and listless than that of 5 days infection. Intestinal contents were greenish, liquid and bulky. Mucosal surface was flat and coarsely granular, showing marked obscuring of transverse folds. Swelling of mesenteric lymph nodes was prominent, measuring up to 1 cm in maximum diameter, as well as enlargement of Peyer's patches.

At the 15th day of infection, serosa of small intestine was smooth and glistening, and the lumen contained bulky and mushy feces. Loss of transverse folds and velvety appearance was not so prominent as that of early phase of infection.

In the 4th and 8th week of infection, serosal and mucosal pattern of the small intestine inclined further towards the normal one. Feces were still loose and mushy, and transverse folds were slightly obliterated. Mesenteric lymph nodes and Peyer's patches were moderately enlarged.

In the 10th week of infection, all features of the small intestine appeared normal, except that loose and soft intestinal content, and enlargement of mesenteric lymph nodes and Peyer's patches lasted as far the end of the experiment.

II. Distribution and density rate of worms in the small intestine

<table>
<thead>
<tr>
<th>Duration of infection</th>
<th>*Point of worm detection</th>
<th>Worms detected in</th>
<th>Total number</th>
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<td>First</td>
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<tr>
<td>8 weeks</td>
<td>1.3</td>
<td>74.2</td>
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*: proximal percentile in small-intestinal length
DR: density rate/1,000/cm
The number of two-strip slides was in the range of 40-50 in each cat. The total number of worms, distribution, and density rate in each part of the small intestine are shown in Table 1 and Fig. 1.

In the light-infection group, the number of sectioned worms varied greatly ranging from 6 to 306 per animal. The point where the sectioned worm was found first lay in proximal 0.5-10.9 percentile of the whole length of small intestine. And the last point where the sectioned worm extended was in the range of proximal 58.2-88.9 percentile of the small intestine. The most proximal point of worm detection was in the level just distal to the ampulla of Vater.

The distribution and the density rate of sectioned worms were quite variable individually, not only by the location of small intestine but also by the infection time. But most worms were found largely in the proximal 60-70 percentile of the small intestine and scarcely seen in the distal ileum.

In the heavy-infection group, the mean number of sectioned worms per animal increased about two times as that of the light-infection group, and the distal end of parasitism extended toward the ileocecal valve in this group. However, similarly to the light-infection group, there was a great individual variation in the distribution pattern.

III. Light microscopic findings

1. Histologic change related to the infected worms

When a *M. yokogawai* worm was found in the slide, an oral portion of the worm faced always toward the submucosa, occasionally being in direct contact to the mucosal cells. In the 5th day of infection, over 50% of the worms located in the lower portion of the inter villous space (Figs. 8 and 9). After the 10th day of infection, the worms were mostly seen in the lowermost part of the inter villous space and some were impacted in the mouth of Lieberkuhn's crypts (Fig. 10). Lining epithelial cells adjacent to the infected worm were markedly compressed and degenerative, associated with more prominent inflammatory cell infiltration in
the surrounding lamina propria (Fig. 14).

In some areas, there were no recognizable lining cells between the worm and the basement membrane, where the basement membrane was occasionally swollen and fragmented. Thus, membraneous or linear structures of the basement membrane were obscured.

Among all of the worms examined, only one reached to the mid-portion of crypt in the proximal ileum at the 4th week of infection. This worm also remained in the glandular lumen, though the lining epithelium of the gland was markedly atrophied together with conspicuous destruction of the basement membrane (Fig. 11).

Three worms were identified in the vicinity of and inside the lymphoid tissue of Peyer's patches in the mid-portion of the distal ileum in the heavy-infection group, at the 4th week of infection. One was found in the mouth of a Lieberkuehn's crypt which was in direct contact to the lymphoid tissue. Another worm was seen just beneath the above, inside lymphoid tissue, but it was covered by degenerative and atrophied epithelial cells with indistinct basement membrane (Figs. 12 and 13). However, no eggs were found in the lymphoid tissue near the worm. The third worm was observed inside lymphoid tissue near the two worms described above, and it was surrounded by rather well preserved glandular structure. Otherwise, worms were never found inside the lamina propria or in the submucosa.

2. General mucosal changes

Mucosal changes were manifested along the whole length of the small intestine and throughout the whole infection period.

In the 5th and 10th day of infection, inflammatory cell infiltration was quite remarkable (Figs. 8 and 15-17). Plasma cells and lymphocytes were the main constituents together with conspicuous eosinophilic infiltration. Neutrophilic infiltrates were also seen occasionally, especially in the early phase of the infection. Sometimes aggregates of the inflammatory cells were found in the lumens of Lieberkuehn's glands intermixed with necrotic tissue debris. Inside the lining epithelium, considerable inflammatory infiltrates were observed, the main constituent of which was lymphocyte (Fig. 17). There were marked edema and capillary congestion in the lamina propria of villi (Fig. 16). Secondary to these changes, villi were profoundly swollen and blunted (Fig. 15). Another remarkable change was shortening and fusion of villi and moderate hypertrophy of crypts (Fig. 15). Goblet cells were markedly reduced in number and the residual amount of cytoplasmic mucous substance was also decreased. This goblet cell change affected the entire small intestine and more prominent in the tip portion of each villus (Figs. 8, 9, and 15). There was no significant difference of these mucosal changes between the light- and the heavy-infection groups.

In the 15th day of infection, inflammatory change of stroma became to be slightly subsided. More remarkable stromal change was marked reduction of capillary ectasia and edema. Thus, villi became less blunt and less fused. But lamina proprial and intraepithelial inflammatory cell infiltration was still moderate together with depletion of goblet cells in the tip portions.

During 4-8 weeks of infection (Figs. 10 and 18), edema and capillary congestion of the stroma and fusion of villi were scatteredly seen. Thus, villi tended to restore the normal appearance except for mild blunting and thickening. But inflammatory cell reaction in the stroma and in the lining epithelium was persistent, though became milder in severity. Goblet cell changes became somewhat mild in the light-infection group, but still remained to be unchanged in the heavy-infection group.
Table 2. Summary of mucosal changes in light-infection group

<table>
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<tr>
<th>Duration of infection</th>
<th>Parts of intestine</th>
<th>Villous pattern</th>
<th>Stromal change</th>
<th>Intraepithelial cell infilt.</th>
<th>Goblet cell depletion</th>
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At the 10th week of infection (Fig. 19), general villous pattern appeared normal except for slight blunting and fusion. Goblet cells became to restore their normal features in number and cytoplasmic mucous amount. But inflammatory cell infiltration in the lamina propria and among the villous epithelial linings remained constantly.

In summary, mucosal changes and goblet cell depletion were prominent during 15 days of infection in both light- and heavy-infection groups, and thereafter, showed tendency of recovery. During 4-10 weeks of infection, general mucosal pattern became normalized, but intraepithelial and stromal inflammatory cell reaction was constantly observed. Restoration of goblet cell depletion appeared rather late than that of villous change, but in the 10th week of light-infection group, goblet cells returned to the normal pattern. In the heavy-infection group, the recovery of these changes seemed to be delayed. Mucosal changes were summarized in Table 2 (light-infection group) and 3 (heavy-infection group).

No recognizable changes were observed in the submucosa or the muscle coat.
Table 3. Summary of mucosal changes in heavy-infection group

<table>
<thead>
<tr>
<th>Duration of infection</th>
<th>Parts of intestine</th>
<th>Villous pattern</th>
<th>Stromal change</th>
<th>Goblet cell depletion</th>
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![Fig. 2](image1.png)

Fig. 2. Villous height-crypt length (V/C) ratios by duration of infection in light-infection group.

3. Crypt length, villous height and villus/crypt (V/C) ratio

Crypt length and villous height were measured in the mid representative portion of each part of the small intestine. Thus, villous height

crypt length (V/C) ratio was calculated and its changing pattern by the duration of infection was illustrated in Figs. 2 and 3, compared to the control one (0 day).

During the first 15 days of infection, crypts...
were evidently thickened in contrast to prominent shortening of villi in both groups. Consequently V/C ratio decreased. These changes were interpreted as villous atrophy and crypt hypertrophy, since luminal distension of Lieberküehn's crypt was not significant.

In the 4th week of infection, these measurements significantly normalized (by Kruskall-Wallis test), and during 8–10 weeks of infection, mucosal pattern was quite similar to that of control group in terms of villous height and V/C ratio.

4. Electron-microscopic findings

In the control group, there was a trend that the height of microvilli was different by the different location of each villus; lowest in the tip portion and highest in the low to mid portion.

During 15 days of infection, microvilli were definitely decreased in height, compared to that of the control group. In the group of 4–8 weeks infection, the height of microvilli was not consistent and varied greatly, although they were generally shorter than the control group.

Microvilli in the early phase of infection were irregular in height and thickness as with loss of covering glycocalyx. This microvillous change was less prominent in the later stages of the infection. Chronological change of microvillous height in different groups are shown from Fig. 20 to Fig. 23.

Observations of villous pattern with scanning microscope were made in the cats killed after 4 weeks of infection. In the 4th week of infection (Figs. 24 and 25), villi were rather irregular in height and thickness. Fusion of villi and flattening of their tips were scatteredly seen in the duodenum and jejunum of the heavy-infection group. Leaf-like picture was not observed. Goblet cells were depleted especially in the jejunum (Fig. 26). In the 8th and 10th week of infection, height and thickness of villi became rather even and leaf-like feature was manifested as seen in the control group (Fig. 27). At these weeks of infection goblet cell depletion was slight in both groups. As other pictures, it seemed that the larger the infected dose, the severer the scanning microscopic findings and the later the restoration of them.

DISCUSSION

As for the locality of *M. yokogawai* in small intestine, Takahashi (1929) and Koga (1938) reported that the upper and middle intestine was the usual site of infection in cats and dogs, although it could extend down to the lower part of the intestine in cats. The locality of parasitism in the human case was reported by Yokogawa (1913), who performed postmortem examination in Formosa and stated that the worm was found most abundantly in the upper and middle parts of the small intestine. Similarly in this study the worms in tissue phase were mostly seen in the proximal 60–70 percentile of the small intestine in the group that was challenged with 10,000 metacercariae, thus supporting the view that the principal locality of parasitism is primarily the upper and middle small intestine in the natural hosts. When infected dose was increased to 50,000 metacercaia, the worms were also found in the distal ileum.

Obliteration of transverse folds might be secondary, as seen in inflammatory bowel disease, to inflammatory process which is represented in this study by the mucosal inflammatory reaction and enlargement of intestinal lymphoid apparatus. Chai (1979) observed luminal dilatation in experimental rat infection and suggested its possible relationship with increased intraluminal pressure. Paralytic process due to inflammation might induce obliteration of transverse folds, further, dilation of the lumen. But luminal distension was never the prominent feature in
this study.

Histologic alterations of the intestine were
counted to the mucosa as were the other reports
(Takahashi, 1929; Koga, 1938; and Chai,
1979). The mucosal changes in the early phase
of metagonimiasis could be summarized as
fusion, thickening, blunting, and shortening of
villi, hypertrophy of Lieberkuhn's crypts, and
goblet cell depletion, together with marked
stromal inflammatory cell infiltration, edema and
vascular ectasia. After the 4th week of infection,
mucosal changes began to be restored, and at the
8th week of infection general mucosal configu-
ration, especially villous pattern appears to be
normal in spite of the parasitism of the worms
in the intervillous spaces. This restoration pat-
tern of the mucosa was reported in no papers
as far as literature is concerned. At present, the
mechanism of the spontaneous restoration could
not be easily explained. One might relate and
extrapolate this phenomenon to human infection.
We are aware of the fact that in endemic areas
of metagonimiasis there are many completely
asymptomatic patients despite the fact of the
persistent and heavy shedding of eggs.

Faust et al. (1968) assumed that heterophyid
trematodes in the mucosal crypts of the duod-
enum and jejunum produce superficial irritation
and necrosis of the mucosal epithelium. Appar-
extly the lining epithelium of the villi near the
infected worm was compressed and destroyed
accompanying more exaggerated inflammatory
change in the adjacent lamina propria, suggest-
ing direct destruction of epithelium by the
infected worm. Thus the early mucosal changes
of metagonimiasis might be explained by me-
chanical destruction of the infected worms.
However, the mucosal alteration in metagoni-
miasis could not be explainable only by me-
chanical trauma because restoration to the normal
villous pattern took place in the later stages
though the worms remain intact in the inter-
vilious spaces. Bulky foamy intestinal content
and increased intraluminal pressure by gas and
mucous content was proposed as one of the
causes of villous atrophy (Chai, 1979). But in
this study their role seems minimal or negligible
in the genesis of mucosal change.

The mucosal changes as seen in metagonim-
iasis often take place in the coeliac disease,
tropical sprue, steatorrhea, megaloblastic ane-
mia, and cholera (Gangarosa et al., 1960). In
many respects, villous changes of Strongyloides
stercoralis infection (Milner et al., 1965), hook-
worm infection (Sheehy et al., 1962; Salem
and Truelove, 1964) and Nippostrongylus bras-
ilienis infection (Jarrett et al., 1968) resemble
those seen in this study, especially in the early
stages. Presumably shortening, thickening and
flattening of villi may represent the waste
basket of abnormal villous condition, and result
in intestinal malabsorption secondary to sub-
stantial loss of absorptive surface area. In fact,
these diseases have been found to be a cause
of malabsorption in man. To assess villous
surface area Rubin et al. (1960), Thurlbeck et
al. (1960), Mandanagopalan et al. (1965), and
Stewart et al. (1967) measured villous height
and crypt length with their ratio arithmetically
and found marked reduction in villous heights
and villous/crypt ratio in malabsorption syn-
dromes. With the similar results as the above,
it could be suggested that in the early phase
of M. yokogawai infection there might be
malabsorption. Furthermore, reduction of micro-
villous height with destruction of glycocalyx
observed in the early phase of infection is
assumed to be related to malabsorption. Support-
ing this suggestion, similar microvillous
change was observed in coeliac disease (Shiner,
1974) and in secondary disaccharidase deficiency
states (Phillips et al., 1980).

Increased turnover rate of intestinal epithelial
cells was reported in various malabsorption
conditions (Laster et Ingelfinger, 1961; Eastwood, 1977) and also observed in some parasitic disease (Da Costa, 1971; Ferguson et al., 1980), where crypt hypertrophy was considered as indirect parameter of the high epithelial turnover. Da Costa (1971) suggested that the high epithelial cell turnover may result in excessive loss of endogenous substances. With the low villus/crypt ratio, evident crypt hypertrophy in the early stages of this study might be interpreted as increased turnover of epithelial cells.

From the above considerations, it might be postulated as a cause of diarrhea in M. yokogawai infection that the worm might have stimulated directly the epithelial cells producing excessive secretion of mucus by mechanical irritation to result in diarrhea. As the trauma proceeds further, changes of villous and microvillous configuration could take place, followed by malabsorption by reduction of surface absorptive area. Also excessive loss of endogenous substances could occur by way of the high epithelial cell turnover and might facilitate diarrhea together with malabsorption.

There might be some debatable points in the above assumption. In the later stages of infection, severity of diarrhea and/or fluidity of the feces was markedly reduced, while worms were persistently observed in the lower-most portion of the intervillous space. However, further study on the biochemical, physiological and immunological characteristics of the worm and host would bring out the more reasonable explanation.

The cause of decrease of goblet cells in number and cytoplasmic mucous content in this study is hardly explainable. It might have been due to early exhaustion and extrusion of secretory vacuoles secondary to mechanical irritation of the infected worm since the worms were largely parasitized in the lower-most portion of the intervillous space. Goblet cells in the Lieberkuehn’s crypts remained to be rather unchanged. In contrast to this, Chai (1979) observed increased goblet cells in the infected rats with M. yokogawai. Gangarosa et al. (1960) reported conspicuous increase of goblet cells within the Lieberkuehn’s crypts and at the base of villi with almost complete absence at the luminal surface of villi in cholera. But proper explanation is not available.

Difficult point of heterophyid trematode infection is the visceral involvement (Africa et al., 1935, 1937, and 1948) such as brain, heart or spinal cord. Faust et al. (1968) mentioned that by the deep penetration of the worms their minute eggs may get into mesenteric venules or lymphatics and are carried to the ectopic sites. No papers are available about the visceral involvement in M. yokogawai infection. However, this possibility can not be completely excluded when the histologic findings observed in this study were considered. Several worms were found in the lymphoid tissue of Peyer’s patches though they were partly surrounded by necrotic and destructive glandular structures. A possibility of delivering of eggs into the adjacent lymphoid tissue and then carrying to the other site could not be ruled out. If it be present in M. yokogawai infection, lymphatics might be the portal of entry rather than venules, since several worms were present in the lymphoid tissue and destructive change of vascular structures was not evident.

**SUMMARY**

To study the basic pathological changes of small intestine in metagonimiasis, light- and electron microscopic studies were made, using a total of 21 cats which were experimentally infected with metacercariae of *Metagonimus yokogawai*. The metacercariae were obtained from naturally infected sweetfish (*Plecoglossus altivelis*) by digestion technique. The cats were
divided into control, light-infection (10,000 metacercariae infected) and heavy-infection (50,000 metacercariae infected) groups. Cats were killed at the 5th, 10th, 15th day, and 4th, 8th and 10th week after the infection. And the small intestine was prepared for the study. Pathological studies comprised gross examination, worm distribution pattern, light microscopic examination and both transmission and scanning electron microscopic examinations.

The results obtained were summarized as follows.

1. Gross morphologic changes were the most marked during the first 2 weeks after infection. The gross abnormalities were severer in the heavily infected animals. The changes were: dryness and listlessness of serosal surface due to dehydration, mushy and/or watery intestinal content, effacement of transverse folds and enlargement of mesenteric lymph nodes and Peyer’s patches. After 4 weeks of infection, these changes became less marked showing a tendency to return to normal.

2. The sectioned flukes were distributed from duodenum to proximal ileum. However, individual variation was marked in distribution. In the heavy-infection group, the locality of parasitism tended to extend more distally.

3. The locality of *M. yokogawai* in the intervillus space was mostly in the lower-most portion of intervillus space, where they compressed and eroded epithelial cells probably due to mechanical damage to the structure. Very rarely the worms were found in the lumen of Lieberkuehn’s crypt, and reaching, in two occasions, into proprial lymphoid tissue.

4. Light-microscopically the lesion was restricted in mucosa: Early mucosal changes were shortening, blunting, fusion, and thickening of the villi, crypt hypertrophy with consequent decrease of villus/crypt ratio, as well as stromal changes of edema, capillary ectasia and marked inflammatory cell infiltration of lymphocytes and plasma cells. Goblet cells were markedly reduced in number as with depletion of its cytoplasmic content. In the later stages of infection, mucosa restored its normal configuration in spite of persistent parasitism of the worms.

5. At the infection stage of 5–15 days, there was significant shortening of the microvillous height with variable destruction of glycocalyx in electron microscopic examination. With lapse of infection time, microvilli became to restore the normal pattern.

With these morphological changes, it appears that diarrhea in experimental metagonimiasis would be related to the decrease of absorptive surface of the small intestine particularly in the early phase of infection. The significant changes seen in villi and microvilli might be due to massive intrusion or invasion of *Metagonimus* worms into the crypts, causing direct mechanical and possible host-immune response to the small bowel mucosa.

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LEGENDS FOR FIGURES

Fig. 4. Serosal surface in a cat of 80,000 metacecercarial infection at the 18th day of infection. Note marked enlargement of mesenteric lymph nodes and apparently dry serosal surface.

Fig. 5. Mucosal surface of control group in jejunum. Transverse folds are well preserved and surface is velvety.

Fig. 6. Mucosal surface of jejunum in preliminarily studied (80,000 metacecercariae) group at the 5th day of infection. Transverse folds are markedly effaced. Note granular pattern presumably due to worm para-
Fig. 7. Mucosal surface of jejunum in preliminarily studied group at the 18th day. Lumen contains foamy watery feces and there is moderate effacement of transverse folds.

Fig. 8. Jejunum of heavy-infection group in the 5th week. Because of marked shortening of villi, a worm is found in the full length of intervillus space. Goblet cells are markedly reduced in number, and depleted. H-E stain, ×40.

Fig. 9. Duodenum of heavy-infection group in the 10th day. Worms are observed in the mouth of Lieberkuehn’s crypts. H-E stain, ×40.

Fig. 10. 4th week, light-infection group. A worm is tightly impacted in the mouth of crypt. Adjacent epithelium is compressed, and goblet cells are moderately depleted. PAS stain, ×100.

Fig. 11. At the 4th week of heavy-infection, one worm is seen in the mid region of Lieberkuehn’s crypt, but within the lumen of gland. H-E stain, ×100.

Fig. 12. Proximal ileum of heavy-infection group in the 4th week. Two worms are seen. One is present in the gland directly contacted with lymphoid tissue, and the other, inside lymphoid tissue. H-E stain, ×100.

Fig. 13. Higher magnification of Fig. 12. In the lower portion glandular structure is entirely destructive. H-E stain, ×400.

Fig. 14. The epithelial cells around the oral sucker of a worm show a marked attenuation and obscured basement membrane, associated with stromal inflammatory reaction. H-E stain, ×360.

Fig. 15. Duodenal mucosa of heavy-infection group in the 5th day. There are marked fusion and shortening of villi together with flattening of their tips. H-E stain, ×40.

Fig. 16. Higher magnification of Fig. 15, showing marked capillary ectasia, edema and inflammatory reaction in lamina propria. H-E stain, ×100.

Fig. 17. Jejunal mucosa of heavy-infection group in the 5th day. Blunting of villi and adhesion are seen, together with stromal edema and inflammatory cells. H-E stain, ×360.

Fig. 18. Jejunal mucosa of heavy-infection group in the 4th week of infection. Villus/crypt ratio is relatively normal and goblet cells are slightly depleted. PAS stain, ×100.

Fig. 19. Jejunal mucosa in light-infection group in the 10th week. Three parasitic worms are seen impacted in the mouths of crypts. H-E stain, ×100.

Fig. 20. Transmission electron micrograph of jejunal microvilli in the mid portion of a villus. Heavy-infection group after 10 days of infection. The microvilli are definitely reduced in height. There is also irregularity of surface plane of individual microvillus with destruction of glycocalyx. ×17,000.

Fig. 21. Jejunal microvilli at mid-villous region in heavy-infection group after 4 weeks of infection. Although less prominent than Fig. 20, there is still reduction of the height. ×17,000.

Fig. 22. Jejunal and mid-villous microvilli in a heavy-infection group after 8 weeks of infection. The height of microvilli is decreased. ×17,000.

Fig. 23. Mid-villous, jejunal microvilli in a light-infection group after 8 weeks of infection. The microvilli become almost completely restored to normal appearance. ×17,000.

Fig. 24. Scanning electron micrograph, showing fusion of villi of jejunal mucosa in a heavy-infection group, 4 weeks after infection. ×360.

Fig. 25. Irregularity of the shape and erosive change at the tips of villi seen in a light-infection group after 4 weeks of infection. ×200.

Fig. 26. Depletion of goblet cells on the surface of the villus shown. Jejunum in a heavy-infection group after 4 weeks of infection. ×750. Inset; ×2,250.

Fig. 27. Leaf-like villous picture in a light-infection group, 8 weeks after infection. ×360. Inset (×1,080); Goblet cell depletion is no longer present.