Morphological Alterations of the Intestinal Villi and Absorptive Epithelial Cells in each Intestinal part in Fasted Chickens

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To demonstrate morphologically the functional difference in each intestinal part, 27 (starter stage)-and 113 (developer stage)-day-old male Single Comb White Leghorn chickens were fasted for 3 or 7 days. After the end of each fasting period, the villous height and the fine structure of absorptive epithelial cells in the duodenum, jejunum and ileum were compared using light and electron microscopies. On the post-hatching developmental changes of the villous height in the normal control, the duodenum had the highest villi at starter stage followed by undeveloped lower jejunal villi, while the latter showed a marked growth rate up to the developer stage. This explains that the vigorous absorptive part would be mainly the duodenum in early life stage and then extend to the jejunum with increasing age. However, after fasting treatment these proximal intestines had thought to have a high absorptive ability revealed a dramatic reduction of the villous height. This suggests that among the intestinal parts, the higher the intestinal absorptive ability during the normal feeding is, the more rapidly the intestinal villous height is reduced by fasting. In fine structural alterations, the epithelial cells in the duodenum had large lysosomal supranuclear vacuoles even after 3 days fasting. On the other hand, by the first 3 days fasting the jejunal cells showed the lamellar bodies thought as a precursor of vacuoles and had typical vacuoles after next 4 days fasting. These suggest that among the intestinal epithelial cells, the higher the cellular absorptive ability during the normal feeding is, the more rapidly the lysosomal vacuoles grow larger in size by fasting.

Compared with the alterations of villous height and ultrastructure of epithelial cells observed in the proximal intestines, the ileum showed an almost constant villous height and no typical vacuoles but well-developed Golgi apparatuses in cells after fasting. These findings lead to the possibility that the ileum appears to be inactive in absorptive function and it might have another specific function in addition to the conventional absorptive function.


Key words : intestinal villi, epithelial cells, fastings, electron microscopy, chicken

Introduction

The morphology and function of the small intestine are known to be altered by its nutritional condition. In comparative gross-anatomical studies on the intestinal length, weight and area in domestic fowls, meat-type fowls such as broilers and Pekin ducks had a well-developed large intestine from the early life stage, while those of White Leghorn were small at 6 weeks old and showed a marked increase up to 20 weeks (ISSHIKI et al., 1992). In fine structural observations, broilers had larger villi than White

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Leghorn from hatching and the size of the intestinal villi in both birds was accomplished within the first 10 days after hatching, in which the duodenum had the largest villi followed by the jejunum (Yamauchi and Isshiki, 1991). Bacteria adhering to the villous apical area were found only in the ileum of 10-day-old broilers (Yamauchi et al., 1990). In nutritional studies, very little absorption of nitrogen (Imondi and Bird, 1965) and lipid (Wissig and Graney, 1968) was observed beyond the jejunum. The proximal intestine absorbed antibodies selectively but the distal one absorbed them non-selectively (Rodewald, 1973; Abrahamson and Rodewald, 1981). These reports suggest that each intestinal part shows obvious differences in the morphology and function and that the morphology of the intestine has a close correlation with its functional activity. However, many problems for the relationships of the simultaneously induced morphological alterations among each intestinal part still remain to be answered due to lack of detailed whole observations of the intestine under the same nutritional experiment.

The aim of the present study was to clarify how morphological alterations would be induced by subjecting chicks to fasting either at the starting or developing stages in life. In this study, simultaneously induced morphological changes of the intestinal villi and absorptive epithelial cells in each intestinal part were compared using light and transmission electron microscopies.

**Materials and Methods**

Eighty newly hatched male Single Comb White Leghorn (Shaver Starcross strain) chicks (*Gallus gallus domesticus*) were obtained from a commercial hatchery. They were reared in a battery-type brooder during the first 25 days and transferred to the larger cages of 7 birds each from 26 to 60 days of age. Birds were kept in the windowless room under the constant lighting from 6:00 to 20:00 h. At 27 days old (starter stage), 20 birds were randomly selected to start the fasting either for 3 or 7 days with free access to water. Remaining 60 chicks were again moved to the individual cage at 61 days old. At 113 days old (developer stage), 20 birds were subjected to the fasting for 3 or 7 days. As a control, intact chicks were given water and standard proprietary diets matched for each growing stage. Three chicks from each fasting group were sacrificed by decapitation at the end of each fasting period, together with their respective age controls. Small intestine was quickly removed and placed in a fixative containing 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). Duodenum was taken from the gizzard to pancreatic and bile ducts; jejunum from the ducts to Meckel's diverticulum; ileum from the diverticulum to ileo-cecal-colonic junction. The middle part of each intestinal region was used for observations. During the first fixative step, each intestinal tissue block for light microscopy was fixed in Bouin's solution and prepared for paraplast embedding. Five-μm cross sections were made and every 10th section was collected to be observed. After staining with hematoxylin-eosin, the villi having the lamina propria were selected for the villous height measurement. One villous height per one section was measured from the villous tip to the bottom excepting the crypt. A total of 30 intestinal villous heights
in each group were measured using Nikon Cosmozone ls image analyzer (Nikon, Tokyo). For electron microscopy, intestinal specimens were obtained from the facted birds for 3 or 7 days and the control matched for 7 days fasting (129 days old) only in the second fasting group. Each intestinal part was slit longitudinally along its entire length and washed away intestinal contents with buffered saline. To prevent the curling outwards of the slit intestine, the edges of the tissue segments were then pinned to paraffin-covered floor in the petri dish containing 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The tissues were fixed in this flattened position for 2 hours at the room temperature. After post-fixation in 1% osmium tetroxide in the same buffer at 4°C, the tissues were processed by routine procedures. Specimens were observed using a Hitachi H-7100 transmission electron microscope (Hitachi, Tokyo) at 75 Kv.

Statistical comparisons between the control and fasted chickens were made by Student's t-test for villous height.

Results

On the height of villi in each intestinal part, the normal control chicks of starter stage had the developed high villi in the duodenum (average of 2223 mm), the undeveloped lower ones in the jejunum (1089 mm) and the lowest villi in the ileum (700 mm) (Fig. 1). Chicks subjected to 3 days fasting showed a remarkable decrease in the

![Graph showing villous height](image-url)

Fig. 1. Villous height of each intestinal part in 3- or 7-day-fasted chickens started from 27 days old and in their matched controls in age. Duodenum shows the highest villi but they are remarkably decreased even after 3 days fasting. Jejunum and ileum are gradually reduced with fasting.

Values represent the mean and SE for 30 measurements.

**: Significantly different from corresponding controls in the same age (P<0.01).

a, b: a is significantly different from b in each intestinal part (P<0.01).
duodenal villous height, but the fasting for next 4 days did not reveal the marked decrease. The height of villi both in the jejunum and ileum showed a gradual decrease with postponement of 7 days fasting. On the other hand, in control chicks of developer stage the jejunal villi markedly developed from those at starter stage (2413 mm in average, 2.21 times higher) followed by the ileal villi (1197 mm, 1.71 times), while the duodenum had slightly increased villi (2659 mm, 1.19 times) (Fig. 2). After first 3 days fasting, the villous heights of the duodenum and jejunum were rapidly reduced and followed by a gradual decrease due to the further fasting. The ileal villous height, however, was almost similar to that of the control even during the first 3 days fasting but a clear decrease was observed in another 4 days fasting.

On the fine structural alterations of the absorptive epithelial cells, the duodenum in 120-day-old normal chicks (Fig. 3A) had tadpole type mitochondria (Mt) and many absorbed nutrients in the vesicles of the smooth endoplasmic reticulum (arrow heads in Fig. 3A). After 3 days fasting, Mt were altered to smaller and oval-shaped ones (Fig. 3B). Vesicles of the smooth endoplasmic reticulum were also shrunk and included no absorbed nutrients. As a characteristic feature, supranuclear vacuoles, which were spherical and contained the various density and size of dense bodies, were appeared (arrows in Fig. 3B). They were seen apparently fusing with each other, resulting in much larger vacuoles with increasing fasting period (Fig. 3C, D). In the large vacuoles, nonuniform contents and large lamellar bodies (myelin figure) were also found.

![Fig. 2. Villous height of each intestinal part in 3- or 7-day-fasted chickens started from 113 days old and in their respective age controls. Compared with 30 days old, a marked development is seen in control jejunum. Villous height shows a remarkable decrease in duodenum and jejunum but no change in ileum after 3 days fasting, the latter shows rapid down after 7 fasting days. See Fig. 1 for statistic explanations.](image-url)
Absorptive epithelial cells of the duodenum in 120-day-old control chickens (A) and in 3 (B)– or 7 (C and D)-day-fasted chickens started from 113 days old. Absorbed nutrients are found in the vesicles of the smooth endoplasmic reticulum (arrow heads in A). Compared with control, the vesicles are shrunk and include no nutrients but well-developed supranuclear vacuoles are seen after 3-day-fasting (arrows in B) and become much larger with next 4 days fasting. All scale bars: 1 μm, ×12,000. D is another example of the vacuoles in 7 day-fasted chickens.
Fundamentally, epithelial cells of the jejunum in the normal control (Fig. 4A) were similar to those seen in the duodenum except the rod type Mt. However, when birds were subjected to 3 days fasting (Fig. 4B), instead of the supranuclear vacuoles the lamellar bodies were frequently observed as a conspicuous morphological alteration (arrows in Fig. 4B; Fig. 4D). After 7 days fasting, the supranuclear vacuoles appeared (Fig. 4C) but they were smaller than those seen in the duodenum of 7 days–fasted chicks. Compared with the duodenum and jejunum, the ileal epithelial cells showed different alterations in morphology. Even fasting for 3 days, the rod type Mt in control (Fig. 5A) did not show a marked alteration (Fig. 5B). On the contrary, well-developed Golgi apparatuses were frequently observed (arrows in Fig. 5B; Fig. 5D). After 7 days fasting, Mt became smaller with high electron density (Fig. 5C). A typical vacuole seen in the proximal intestinal part was not observed.

Discussion

Intestinal morphological differences among chicken breeds during the post-hatching development reflect their absorptive functions. Meat-type fowls had a well-developed large-sized intestine from early life stage, while that of White Leghorn was small at 6 weeks age and increased markedly up to 20 weeks (ISSHIKI et al., 1992). In the fine structural observations, the broiler intestine had larger villi than that of White Leghorn from hatching and the size of the villi was accomplished within the first 10 days after hatching, in which the duodenum showed the largest villi (YAMAUCHI and ISSHIKI, 1991). The size of villi was demonstrated to be related to the absorptive function (YAMAUCHI and ISSHIKI, 1991, YAMAUCHI et al., 1993) and to be varied with cell mitotic activity of epithelial cells (YAMAUCHI et al., 1993). Villous height also reported to have significant correlation with the intestinal absorptive function in rats (DOWLING and BOOTH, 1967) and to be dependent on the number of epithelial cells in chicks (NODA, 1979). These investigations suggest that the villous size and height are regarded as a useful index to express the intestinal function. At the starter stage in this study, the duodenum had the highest villi. However, up to the developer stage the jejunal villous height showed a marked growth rate. These seem to explain that the vigorous absorptive part would be mainly the duodenum in early life stage and then extends to the jejunum with increasing age.

Marked abnormalities have been reported in the intestinal villi after fasting. In fasted mice for 3 or 4 days, BROWN et al. (1963) demonstrated the following abnormalities: reductions of cell renewal and cell migration rate to the villous tips; vacuolated cuboidal cells rather than columnar cells; cell extrusion into the crypt lumen prior to migrate to villi; less DNA synthesis. The shortening of the villi by our present fasting seems to be induced by these abnormalities described above. Besides, the shrinkage of cells and the atrophy of the cell organella were observed. Although, in the case of the increasing of the villous size (YAMAUCHI et al., 1993) and height (NODA, 1979) an increased cell mitosis has been interpreted as the main factor for their increases, the shrinkage of individual cell is also thought to be an important factor for the shortening of the villous height. PEER et al. (1984) investigated that skip-a-day
Fig. 4. Absorptive epithelial cells of the jejunum in 120-day-old control chickens (A) and in 3 (B and D)- or 7 (C)-day-fasted chickens started from 113 days old. One can see only lamellar bodies thought as a precursor of the vacuole after 3 days fasting (arrows in B; D). Typical vacuoles are seen after 7 days fasting. Scale bars in A–C; 1μm, ×12,000. D is a higher magnification of B (scale bar; 0.5μm, ×37,500).
Fig. 5. Absorptive epithelial cells of the ileum in 120-day-old control chickens (A) and in 3 (B)- or 7 (C and D)-day-fasted chickens started from 113 days old. Well-developed Golgi apparatuses are found after fasting (arrows in B; D) but no typical vacuoles are seen even after 7 days fasting. Scale bars in A–C: 1μm, ×12,000. D is a higher magnification of another example of the Golgi apparatus in 7-day-fasted birds (scale bar: 0.5μm, ×37,500).
feed restriction from 3- to 18-week-old gave no changes to the jejunal mucosal appearance but caused the extensive damage to the duodenum of broilers. This suggests that the degree of the morphological damage due to fasting for the villi may be different among each intestinal part. In this study, a dramatic reduction of the villous height was caused by fasting treatment only in the duodenum and jejunum which had thought to have a high absorptive ability under the normal nutritional conditions. It seems that among the intestinal parts, the higher the intestinal absorptive ability during the normal feeding is, the more rapidly the intestinal villous height is reduced by fasting.

Compared with the fine structure of epithelial cells in the control, the supranuclear vacuoles including dense bodies were frequently observed as a conspicuous morphological feature in fasted chickens. Such vacuolization of the cytoplasm has already reported in mouse jejunal villi after 4 day of starvation (BROWN et al., 1963). The dense bodies in the vacuoles have been demonstrated as secondary lysosomes by means of acid phosphatase staining in the previous study (YAMAUCHI and ISSHIKI, 1994). After food restriction for 8 days, MICHAEL and HODGES (1973) described an elevated activity of acid phosphatase in the slightly shortened villi in cockerel intestine. The lysosomal nature in vacuoles has suggested a digestive function (CORNELL and PODYKULA, 1969). Lysosomes have 3 intracellular digestions such as heterophagy, autophagy and chroinophagy, and these functions regulate the growth, nutrition and differentiation of cells (BAINTON, 1981). In the intestinal epithelial cells showing an atrophic absorptive function, the small-sized lysosomal dense bodies were observed in the chicks reared under the low nutritional conditions (YAMAUCHI and ISSHIKI, 1994). By the present fasting treatment, the large-sized vacuoles including the various density and size of dense bodies appeared. The literatures described above and the present result lead to consider that the large-sized supranuclear vacuoles are developed from the lysosomes due to the lysosomal intracellular digestion after fasting. It was also observed that the large-sized vacuoles were induced by fasting treatment only in the cells in the intestine which had thought to have a high absorptive function under the normal nutritional conditions. It seems that among the intestinal epithelial cells, the higher the cellular absorptive ability during the normal feeding is, the more rapidly the lysosomal vacuoles grow larger in size by fasting treatment. In our another experimental series (unpublished), the development of vacuoles was slower in fatty adult hens than that of lean cockerels. Besides, the large-sized vacuoles seen in the hens fasted for 20 days were rapidly reduced their sizes to the small-sized lysosomal dense bodies by feeding for one day. These may be speculated that the development of lysosomal vacuoles may correlate well with the cellular nutritional conditions.

In the ileum of rats, the following specific functions have been reported. Proteins including antibodies were selectively absorbed from the proximal intestinal cells and those failed to be absorbed there were nonselectively degraded in the distal intestinal cells of newborn rats (RODEWALD, 1973; ABRAHAMSON and RODEWALD, 1981). Besides, the enlarged small intestinal diameter and villous height, and the enhanced glucose absorption were demonstrated in the intestinal remnant following the another intesti-
nal resection in the rats (DOWLING and Booth, 1967). These compensatory changes were found to a much greater in the ileum after proximal resection than in the jejunum following distal resection (DOWLING and Booth, 1967). The duodenum and initial segment of the jejunum absorbed the lipid, while the ileum did not play a significant role in digestion and absorption of triglyceride (Wissig and Graney, 1968). The intestinal physiological characteristic features have been demonstrated also in the chicken ileum. Ingested nutrients were absorbed in the upper part of the intestine (Isshiki et al., 1989). Very little nitrogen absorption was observed beyond the jejunum in young chickens (Imondi and Bird, 1965). This is well in harmony with the morphological characteristic features that bacteria adhering to the intestinal epithelial cells were observed only in the ileum (Yamauchi et al., 1990). In this study, the ileum revealed different morphological changes from those of the proximal intestines; namely, the lowest villous height during post-hatching development, gradual or almost no decrease of it after fasting, no typical supranuclear vacuoles and well-developed Golgi apparatuses. Michael and Hodges (1973) demonstrated the similarity of the structural and functional changes of the fowl intestinal mucosa following restricted feeding to those of the mammals. A consideration of the present results and the findings of similar studies in the literature on mammals leads to the general conclusion that the ileum appears to be inactive in absorptive function and that it might have another specific function in addition to the conventional absorptive function. Since the observations conducted in this study were not sufficient to describe the other sort of ileal function, further study is being carried out in this direction.

In conclusion, the most vigorous absorptive part would be mainly the duodenum in starter stage and then extend to the jejunum with increasing age. These proximal parts revealed the rapid responses in a reduction of their villous heights and in a development of their cellular vacuoles by fasting treatment. Besides, the ileum appears to be inactive in absorptive function.

References


絶食鶏の腸管各部位における絨毛および吸収上皮細胞の形態学的変化

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腸管各部位の機能的差異を形態学的に立証するため，27日齢（幼鶏期）および113日齢（大鶏期）の単冠白色レグホン雛雄鶏を3日間または7日間絶食させ，各実験期間終了後，十二指腸，空腸および回腸における腸管絨毛の高さと呼吸上皮細胞の微細構造の変化をそれぞれ光学顕微鏡および電子顕微鏡を用いて比較検討した。正常飼育対照鶏における絨毛高の孵化後の発育に関しては，幼鶏期では十二指腸が最も発達した高い絨毛を有していた。また，大鶏期にかけて空腸が最も顕著な発達を示したことから，鶏の発育初期における顕著な栄養素の呼吸部位は十二指腸であり，発育と共に空腸へも移行するものと思われる。しかしながら，このような高い吸収機能を有する腸管近位部の絨毛高は，絶食処理により急激な減少を呈した。このことから，腸管各部位のうち正常飼育下で腸管の吸収機能が高い部位ほど，絶食により絨毛高が急激に減少するものと考えられる。吸収上皮細胞における微細構造の変化では，十二指腸の細胞は最初の3日間の絶食により，すでに核上部にライソゾーム由来の巨大空胞を有していた。絶食における最初の3日間の絶食では，空胞の前駆体と思われる層板小体が認められただけであった。しかしながら，次の4日間の絶食により顕著な空胞が出現したことから，吸収上皮細胞のうち正常飼育下で吸収機能が高ければ高い細胞ほど，絶食によりライソゾーム由来の空胞が急速に発達することと推察される。

腸管各部位における絨毛高や上皮細胞の微細構造の変化と比較して，回腸では絶食による顕著な絨毛高の変化はなく，また典型的な空胞の代わりに比較的よく発達したプルジ装置が出現していたことから，回腸は通常の吸収機能には余り関与せず，それ以外にも特殊な機能を有しているのではないかと推察される。

キーワード：腸絨毛，上皮細胞，絶食，電子顕微鏡，鶏