Tropical Medicine, 12 (4) : 179–208, February, 1971

Characteristics of the Intestine of Infantile Mice with Reference to the Pathogeny of Experimental Cholera

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(Received for Publication December 21, 1970)

Abstract

In the pathogenesis of infectious diseases, a certain host factor should play a significant role as well as a pathogenic microorganism. Cholera, an infection with Vibrio cholerae, is not an exception. The clinical manifestation of cholera should be influenced by a certain condition of the host infected with Vibrio cholerae.

In the experimental cholera of mice by oral challenge with Vibrio cholerae which has been developed by Ujiie and others, the incidence of the disease is closely related to the age of mice; only infantile mice younger than one week old of age are susceptible to oral challenge with Vibrio cholerae, and in mice older than one month, neither symptom nor multiplication of Vibrio cholerae in the intestine is seen. Therefore, it is understood that the infancy of mice should be one of the host factors as far as the experimental cholera in mice is concerned.

It is presumed that characteristics of the infancy of mice may consist of uncompleted intestinal flora, deficiency of digestive enzymes, simplicity of intestinal content, particular immunological condition, abnormal absorptive ability of the intestine, etc. In this study, the morphological structure, histochemical behavior and function of the intestinal mucosa were investigated to compare the differences between infantile and adolescent mice.

The characteristics of the infancy were as follows.

1). The surface of villi was lacking in enzyme layer.

2). The intercellular space in the epithelium was wide and conjunction between the cells is lax except apical region.

3). There were a lot of vesicles and vacuoles of various size or phagosomes and

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pinocytotic indentations in the upper part of the absorptive cells.

4). A large number of phagolysosomes were found in the middle part of the cytoplasm.

5). The plasma membrane of microvilli was lacking in trilaminal structure.

6). Polysaccharides and alkaline phosphatase were not or poorly found in the brush border.

7). Epithelial cells ingest protein (ferritin) without decomposition.

The above mentioned characteristics which were found in the infantile mice suggest that toxin or toxin-like substances produced in the intestine by multiplying Vibrio cholerae will be able to be absorbed through the intestinal wall.

So far, no exact knowledge on the host factor in human cholera has been obtained. However, it can be presumed that some of the factors which were indicated by the present study may be a homologous condition in the pathogeny of human cholera.

Introduction

In the pathogenesis of infectious diseases, a host factor should play an important role to reveal a clinical manifestation as well as a pathogenic microorganism. However, very few knowledge on the host factor for establishing the infection has been obtained, while the pathogenicity of microorgnism has been clarified. Cholera, an infection with Vibrio cholerae, is not an exception. There are various clinical manifestations of the infection with Vibrio cholerae, as profuse diarrhea with severe dehydration, moderately severe diarrhea, mild diarrhea and symptomless carrier, even though infections have been caused by a same strain of Vibrio cholerae. The difference of clinical symptom should be due to certain host factors which have remained completely unknown.

In the experimental cholera in mice by oral challenge with Vibrio cholerae which has been developed by Ujiie and others[1], it has been noticed that only infantile mice about one week old of age were susceptible to oral challenge with Vibrio cholerae, and in adolescent mice, neither disease nor multiplication of vibrio in the intestine was found. In other words, the susceptibility of mice for experimental cholera was closely related to the age of the animals, and it has been understood that the infancy should be one of host factors, as far as the experimental cholera in mice is concerned.

It is presumed that characteristics of the infancy related with the pathogenesis of cholera may consist of various elements, for instances uncompleted intestinal flora[2], deficiency of enzymes, simplicity of intestinal content, particular immunological condition and abnormal absorptive ability of the intestine etc. In the present study, the absorptive ability which should be most significant for the pathogenesis of cholera was investigated, comparing the morphological structure, histochemical behavior and absorption between infantile and adolescent mice.

Materials and Methods

Materials

Mice of ICR strain were used for the experiments. They were classified into two age groups, infantile and adolescent; twenty-three mice about a week old of age belonged to the former and twenty-four about a month old to the latter, and two newborns were used as control.

Methods

Epithelial absorptive cells of the small intestine of mice were investigated morphologically, functionally and histochemically in comparison between infantile and adolescent group.

1. Light microscopic examination

Specimens of the small intestine were fixed in ten percent formalin solution. Histological sections were stained ordinarily by hematoxylin and eosin (HE). Periodic acid-Schiff (PAS), alcian blue and oil-red-O were used for staining in the histochemical examinations.

2. Electron microscopic examination

Specimens were fixed just after removal in chilled 2.5 percent glutaraldehyde for one to two hours, and successively fixed in 1 to 2 percent osmium tetroxide for two hours. Both fixatives were phosphate-buffered in pH 7.2. Embedding was in epon after dehydration by ethanol and then aceton. Ultra-thin sections of the specimen were doublestained by ulanyl acetate and lead nitrate.

3. Histochemical examinations

i) Alkaline phosphatase

Tissues were fixed immediately in 2.5 percent cacodylate-buffered glutaraldehyde for two hours. After the fixation, small blocks of the tissue were washed in the buffer for two to four hours and then incubated in reaction medium by Hugon[3] for ten minutes at room temperature. The sections were then briefly rinsed, postfixed in 2 percent cacodylate buffered osmium tetroxide for one hour, dehydrated and embedded in epon.

Control experiments were carried out on substrate-free medium and heated materials after prefixation at 60°C for one hour.

ii) Polysaccharides

The distribution of polysaccharides was traced by PAS-and alcian blue staining under the light microscope.

iii) Lipids

Lipid staining was done by oil-red-O for the light microscopic examination.

4. Absorption test of ferritin

Ten percent ferritin (cadmium free) solution was injected directly into the small intestine. Ten minutes later a piece of the intestine was removed and fixed according to the above-mentioned procedure. The distribution of ferritin in the columnar absorptive cells were observed by the electronmicroscopic examination.

5. Feeding experiment with fat diet

Findings of villi of the small intestine in adolescent mice especially fed with fat diet like cheese were investigated by light and electron-microscope in comparison with those in infantile mice fed with mother's milk.

Result

Morphological findings

1. Light microscopic observation

The size of villi in the infantile group was considerably small and crypts were shallow, compared to the adolescent, although individual epitheliel cells showed similar size with the adolescent. It should be due to the fewer number of epithelial cells in an individual villus of infantile mice than those of adolescent mice. Vacuoles were found in cytoplasm which seemed to be a loose structure in the infantile, cytoplasm in the adolescent presented a dense appearance without vacuoles (Photo. 22 and 23).

2. Electron microscopic observation

In the observation by electron microscope which was done comparatively in the infantile group with the adolescent, the following differences were recognized.

i) Surface coat (Enzyme layer)

The surface coat covering the brush border which is called as enzyme layer was found in the majority of adolescent mice, while it was lacking in the infantile group (Photo. 3 and 5).

ii) Intercellular space of the epithelium

The structure of the epithelium in the adolescent group showed that epithelial cells were regularly arranged with closed intercellular junction, and the interdigitation of each cell membrane was found. In infantile mice, however, the intercellular junctions were lax and frequently opened with space (intercellular space), although the apical part of the cell membrane was tightly closed with desmosome (Photo. 2 and 4).

iii) Vacuoles and pinocytotic indentation

It was noticed in the infantile group that a lot of vacuoles and marked pinocytotic indentations were recognized in the apical cytoplasm with numerous vesicles and small tubulous structure. Contents of vacuoles were not similar; some of them were empty, others were containing small masses or homogeneous substance of low density (Photo. 1, 2 and 3, 3-A, B, C, D).

In the adolescent group, however, the apical cytoplasm presented an appearance of homogeneous structure which contained neither vacuoles nor pinocytotic indentation nor small tubulous structure (Photo. 4 and 5).

Observing the absorptive cells of newborn, pinocytotic indentation, vacuoles and vesicles can be seen in the apical cytoplasm without content and of fewer number than those in the infantile. They are free from lysosomal action (Photo. 8 and 9).

iv) Lysosomes

The supranucleal region of cytoplasm in the infantile group showed the structure containing numerous electron dense and irregularly shaped masses which belonged mainly to lysosomes, especially phagolysosomes. Primary lysosomes were scarecely seen (Photo. 1 and 2). In newborn mice, however, lysosomes belonged mostly to primary lysosomes (Photo. 8 and 9).

On the other hand, in the adolescent group, there were no distinct difference of structure between the apical and subnucleal region of cytoplasm which contained a few primary lysosomes (Photo. 4).

v) Endoplasmic reticulum

Generally there were two kinds of endoplasmic reticulum, smooth and granular. In the infantile, the majority of them appeared smooth and few granular, while

Photo. 1

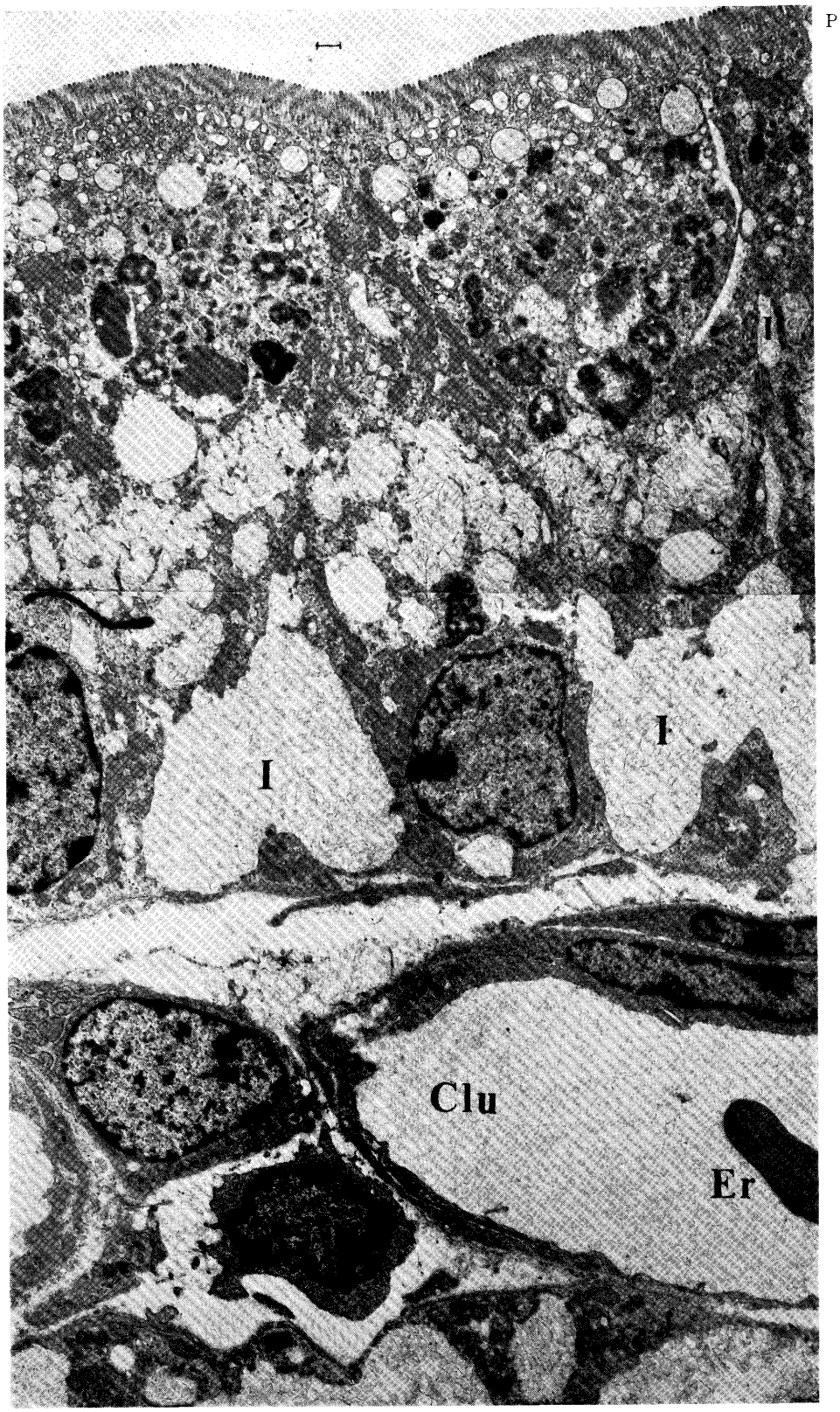


Photo. 2

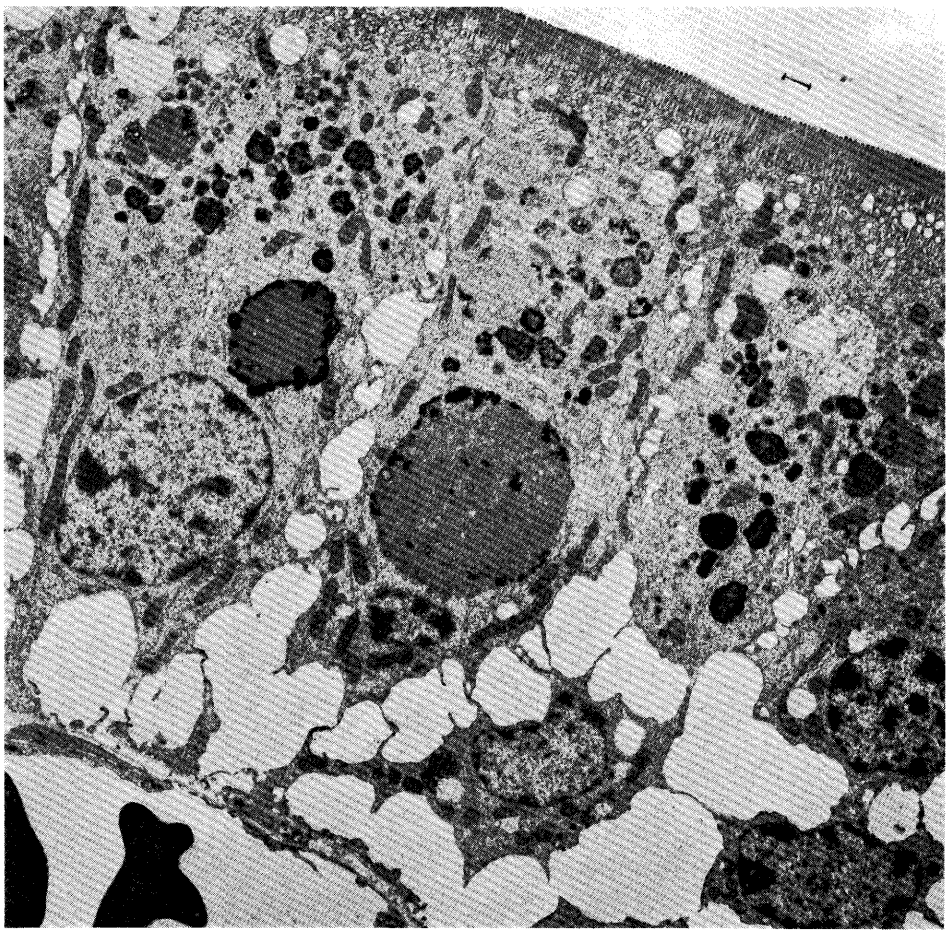


Photo. 1 : General view of the epithelium of an intestinal villus from infantile, 8-day-old, mouse. Apical cytoplasm is filled with a lot of vacuoles varying in size and tubulous structures. Beneath the apical region, numerous lysosomes with high density and with irregular shapes are seen. Most of them belong to phagolysosmes. Following the region with numerous lysosomes, the cytoplasm is widely occupied by reticular structure of low density; presumably fat takes a main part of the region. The similar reticular structure can be seen in the opened intercellular space. It should be noticed that the cytoplasm consists of three zones with diffrent appearance. x3600, I : intercellcular space, Clu : capillary lumen, Er : erythrocyte

Photo. 2 : General view of the epithelium of an intestinal villus from infantile, 8-day-old, mouse. Several absorptive cells with prominently opened intercellular sprce are seen. In the apical region, however, the intercellular juntions are tight. x3900

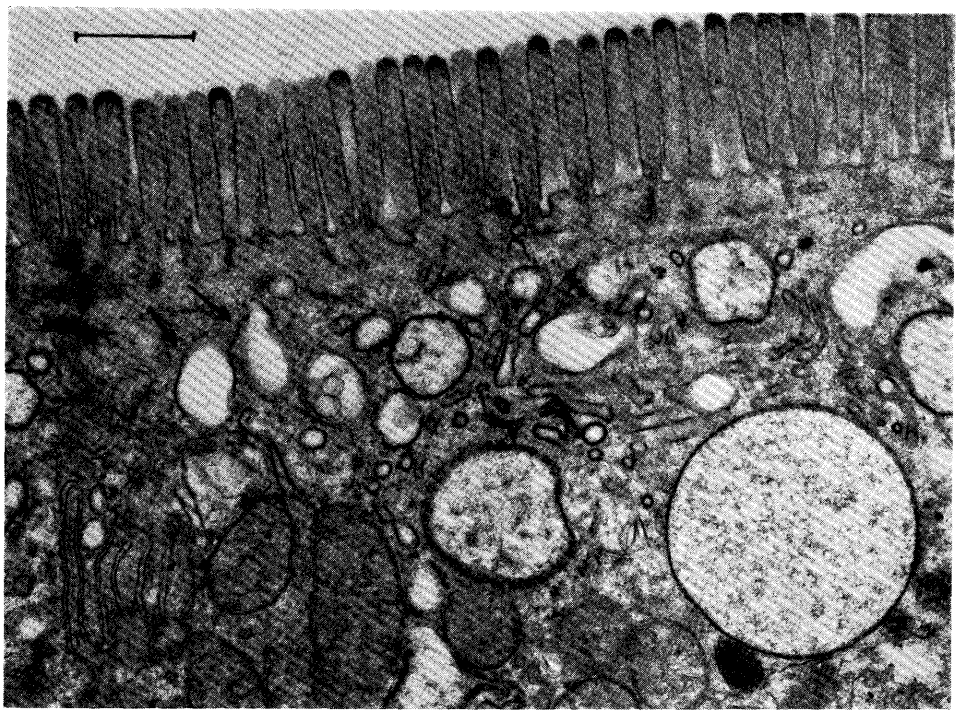


Photo. 3

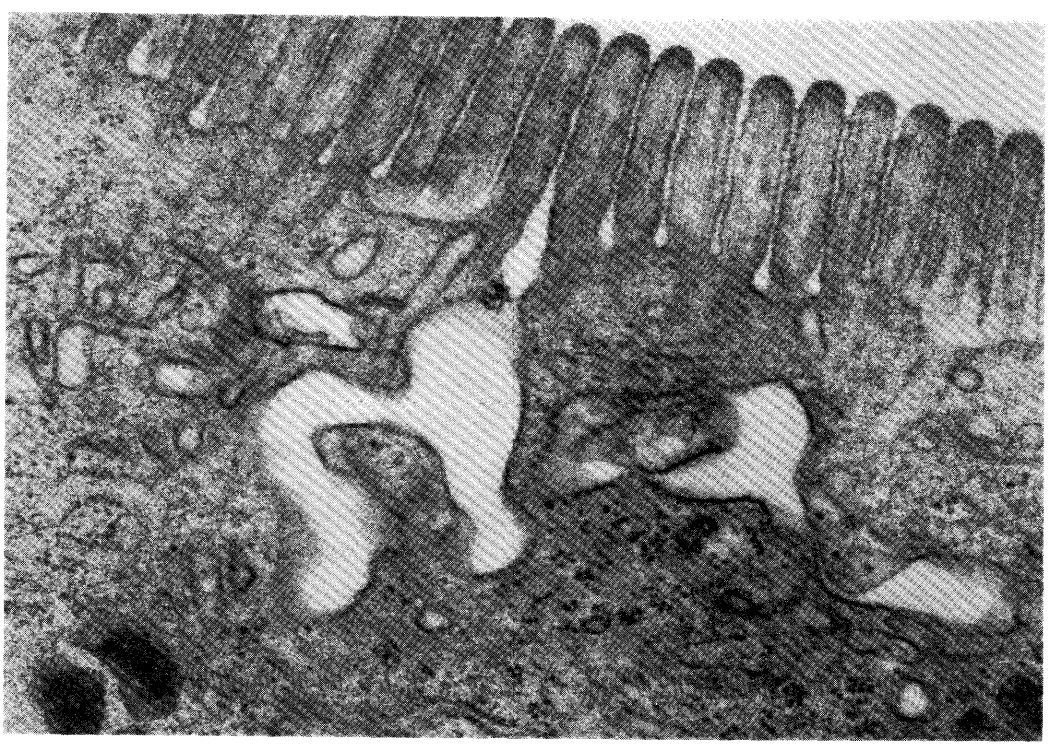


Photo. 3—A

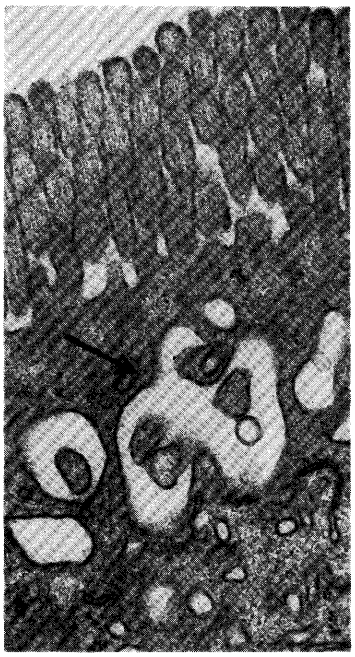


Photo. 3−B

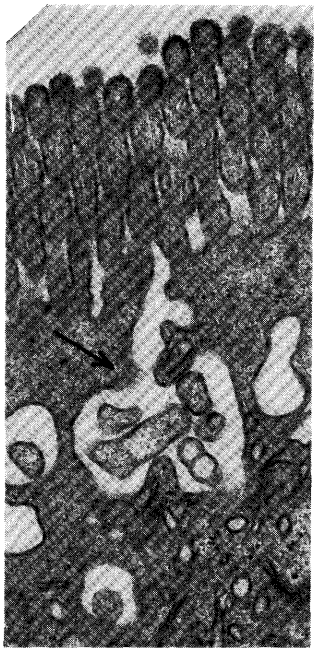


Photo. 3−C

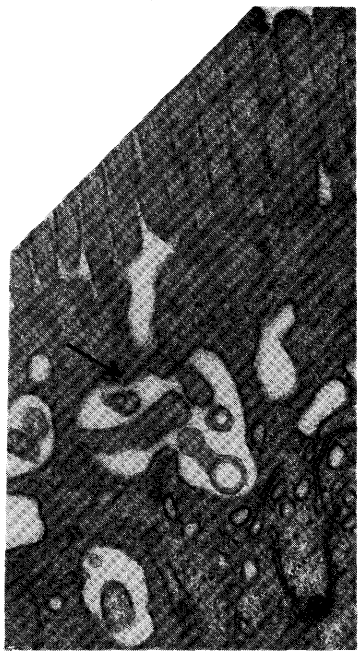


Photo. 3−D

Photo. 3: Apical region of the absorptive cell from infantile, 8-day-old, mouse. Many vacuoles are various in size and with various contents. Tubulous structure among them are presumably smooth endoplasmic reticulums. A few pinocytotic indentations (arrow) are seen, although they are not opened to the intestinal lumen in this picture. Photo.3-A showes a marked pinocytotitic indentation. B, C and D are the pictures of serial sections. In photo.B, what is pointed out by an arrow seemes to be closed containg some substance. But in photo. C, it is proved to be opened into the intestinal lumen. And in photo. D, it is closed. Besides, it is evident that the cytoplasm protrudes into the cavity. x15400 (3-A : x44000, 3-B, C and D : x24000)

in the adolescent, mostly granular and few smooth.

vi) Microvilli

Microvilli of intestinal mucosa in the adolescent group had plasma membrane of which the trilaminal structure was clearly distinguished. The density appeared to be higher in the periphery and lower in the central part. In the infantile group, trilaminal structure of the plasma membrane was not evident and the electron density was homogeneous in the periphery and central part of microvilli (Photo.6 and 7).

Photo. 4

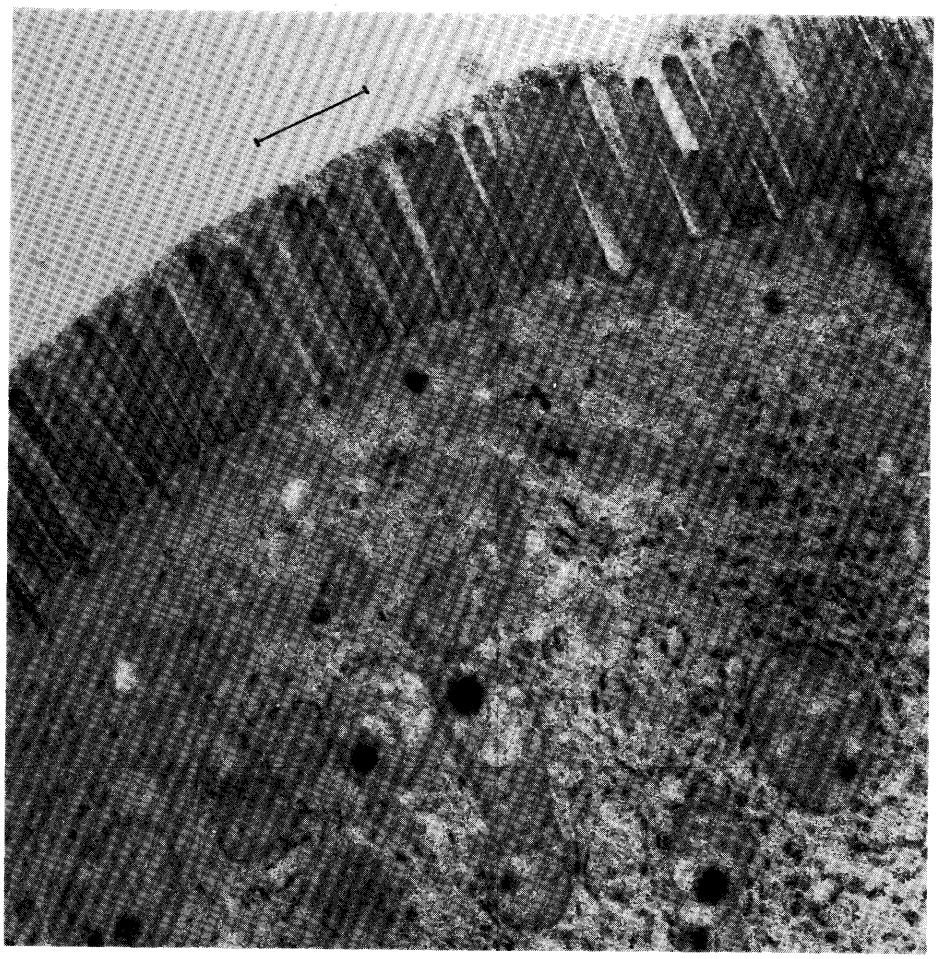


Photo. 5

Photo. 4 : A few columnar absorptive cells of the intestinal epithelium from adolescent, 30-day-old, mouse. Each cells are tall and thin. The composition of the cytoplasm is homogeneous on the whole except the gathering mitochondria in basal region. Intercellular conjunctions are tight from tip to base. Aly : autolysosome, L : lysosome (primary), G : Golgi apparatus, Clu : capillary lumen. x8000

Photo. 5 : Apical part of columnal absorptive cell of the intestinal epithelium from adolescent, 28-day-old, mouse. Brush border is covered by enzyme layer. The cytoplasm contains a lot of granular endoplasmic reticulums x15400

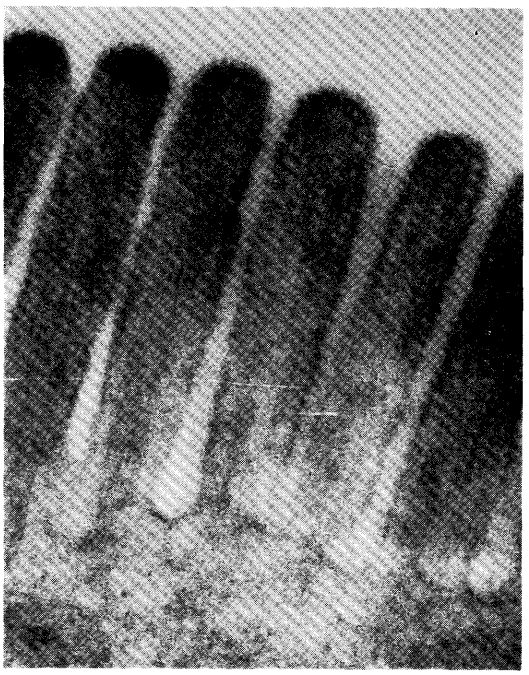


Photo. 6 : Microvilli of the intestinal absorptive cell from infantile, 8-day-old, mouse. The microvilli are homogeneously electron dense and the trilaminal structure of the plasma menbrane is not clearly distinctive. x66000

Photo. 7 : Microvilli of the intestinal absorptive cell from adolescent, 27-day-old, mouse. Trilaminal structure of the plama mombrane is clearly distinctive. x66000

Photo. 8 :
Photo. 9 : Intestinal absorptive cells from new born mice. The tissues were fixed within 10 minutes after birth, before taking nothing by month. Many lysosomes presenting in photo. 8 are primary lysosomes in almost all. Only few secondary lysosomes, presumably being autolysosomes can be seen. Glycogen granules are frequently observed (photo. 9). The findings of the apical cytoplasm is clearly distinguishable from that of a week old mouse. photo. 8 : x4300, photo. 9 : x34000

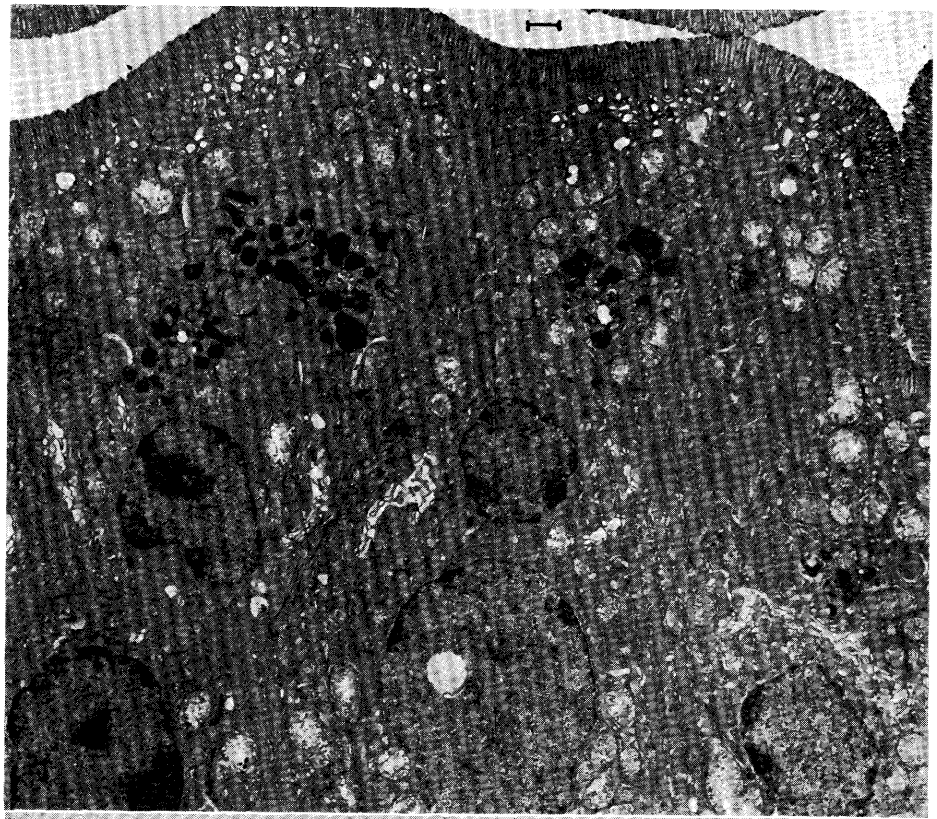


Photo. 8

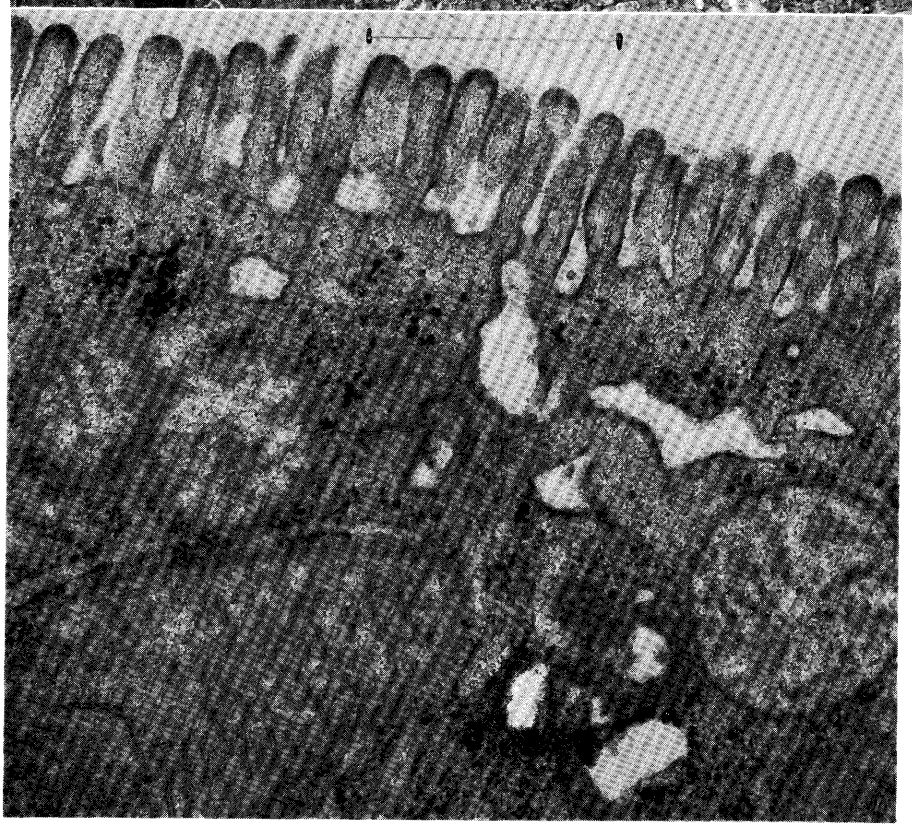


Photo. 9

Histochemical behavior

1. Alkaline phosphatase

It was usually found in the adolescent group that prominent alkaline phosphatase activity was proven on the whole surface of microvilli including surface coat, and precipitation of lead phosphate indicating the activity of alkaline phosphatase was also found in some organelles in the apical cytoplasm (Photo. 10. 11 and 12). Control trials using heated materials and substrate free medium showed no precipitation of lead phosphate in the intestinal mucosae (Photo. 13 and 14).

In infantile mice, however, activity of alkaline phosphatase was scarecely or not proven anywhere in the mucosa of the intestine (Photo. 15 and 16).

2. Polysaccharides

In the adolescent group, the distribution of polysaccharides was proven in goblet cells of the epithelium and brush border of the columnar absorptive cells by means of PAS and alcian blue staining. No positive substance for these stainings was found in cytoplasm of the columnar absorptive cells and lamina propria mucosa of villi (Photo. 26). On the other hand in the infantile group, polysaccharides were proven only in goblet cells, not in the brush border (Photo. 27)

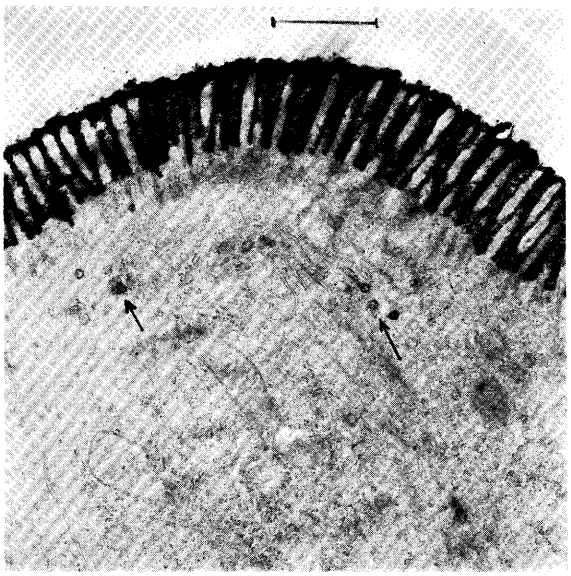


Photo. 10

Photo. 10
Photo. 11 : Alkaline phosphatase activity in the intestinal absorptive cells from adolescent, 32-day-old, mice. Brush border of the cells including enzyme layer has a remarkable activity of the enzyme; and lead phosphate precipitation is also seen in some organelles of the apical cytoplasm (arrow). (Scale showes one micron.)

Photo. 12 : Transverse section of microvilli of the same specimen to that of photo. 11. Left lower part of the picture showes the terminal web.

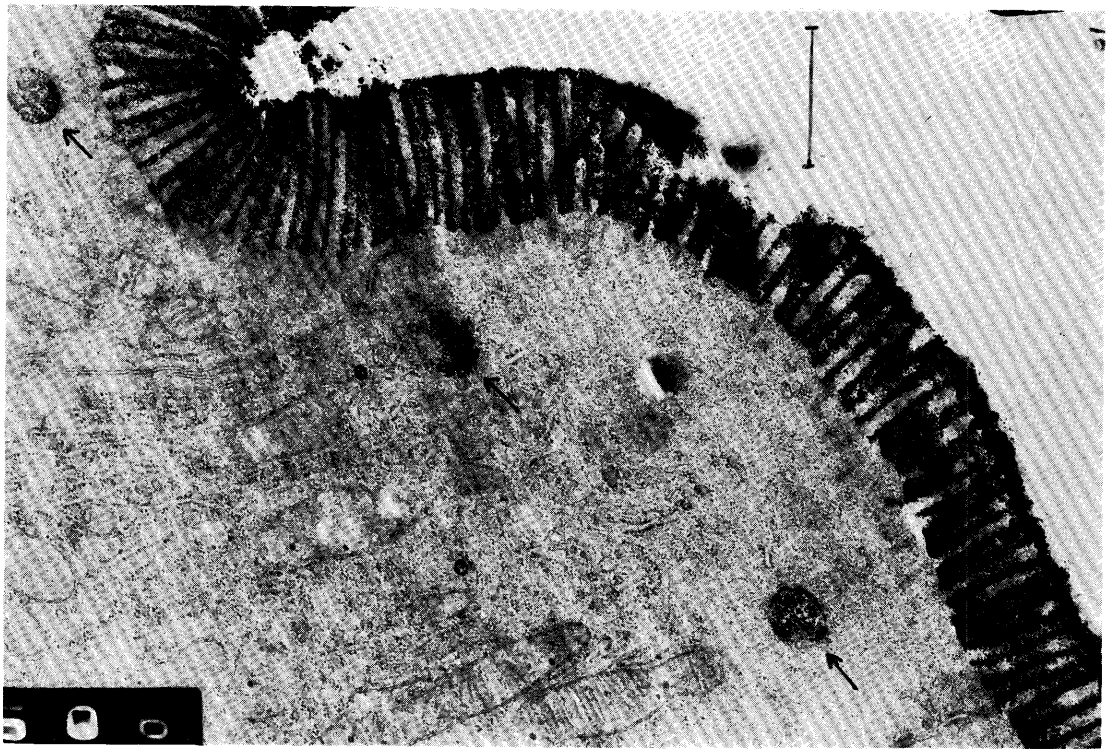


Photo. 11

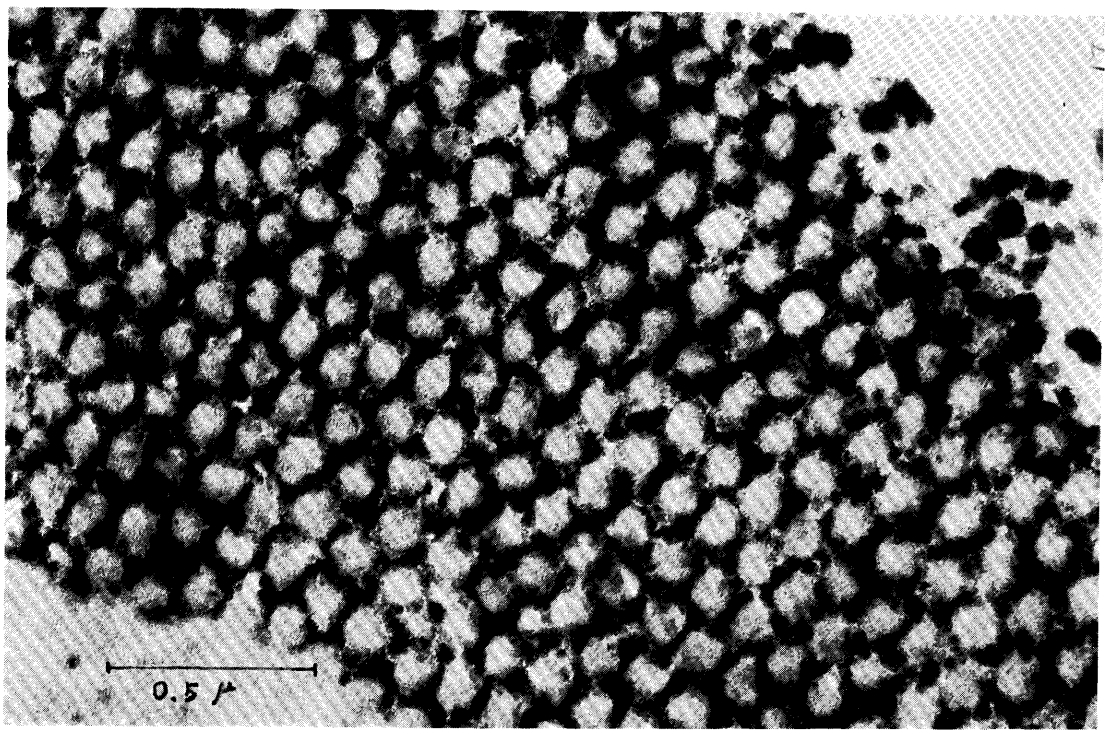


Photo. 12

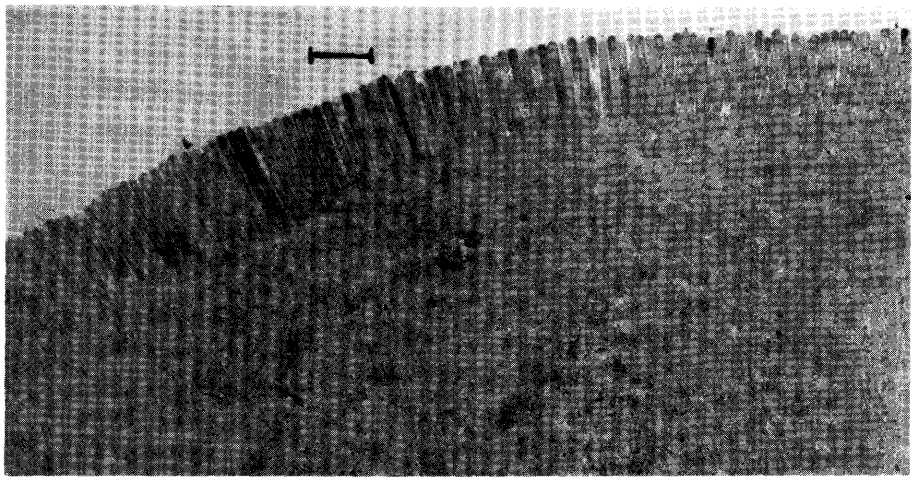


Photo. 13 : Control finding of the same specimen with that of photo. 11. The enzymal reaction was carried out in substrate free medium. Percipitation of lead phosphate is not seen. x8800

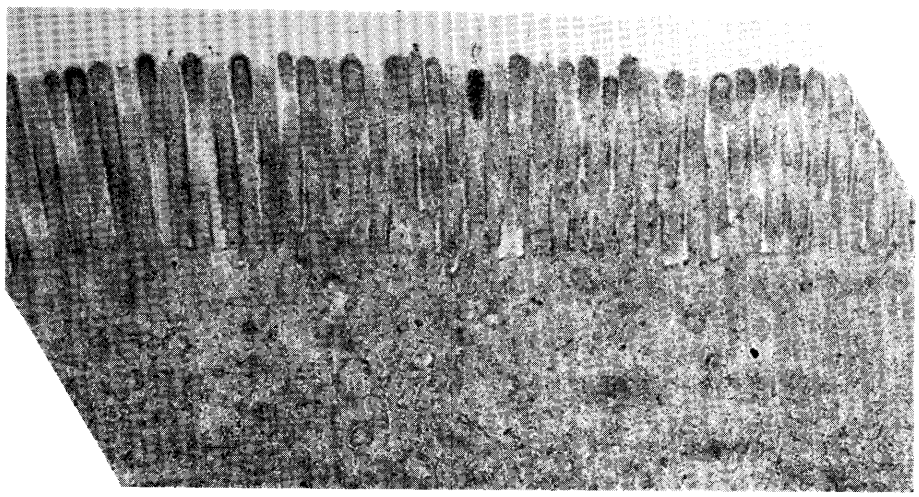


Photo. 14 : Control finding of the same tissue with that of photo. 11. The enzymal reaction was carried out after heating the tissue at 60°C for one hour. No precipitation of lead phosphate indicates that the activity of alkaline phosphatase is lost. x22000

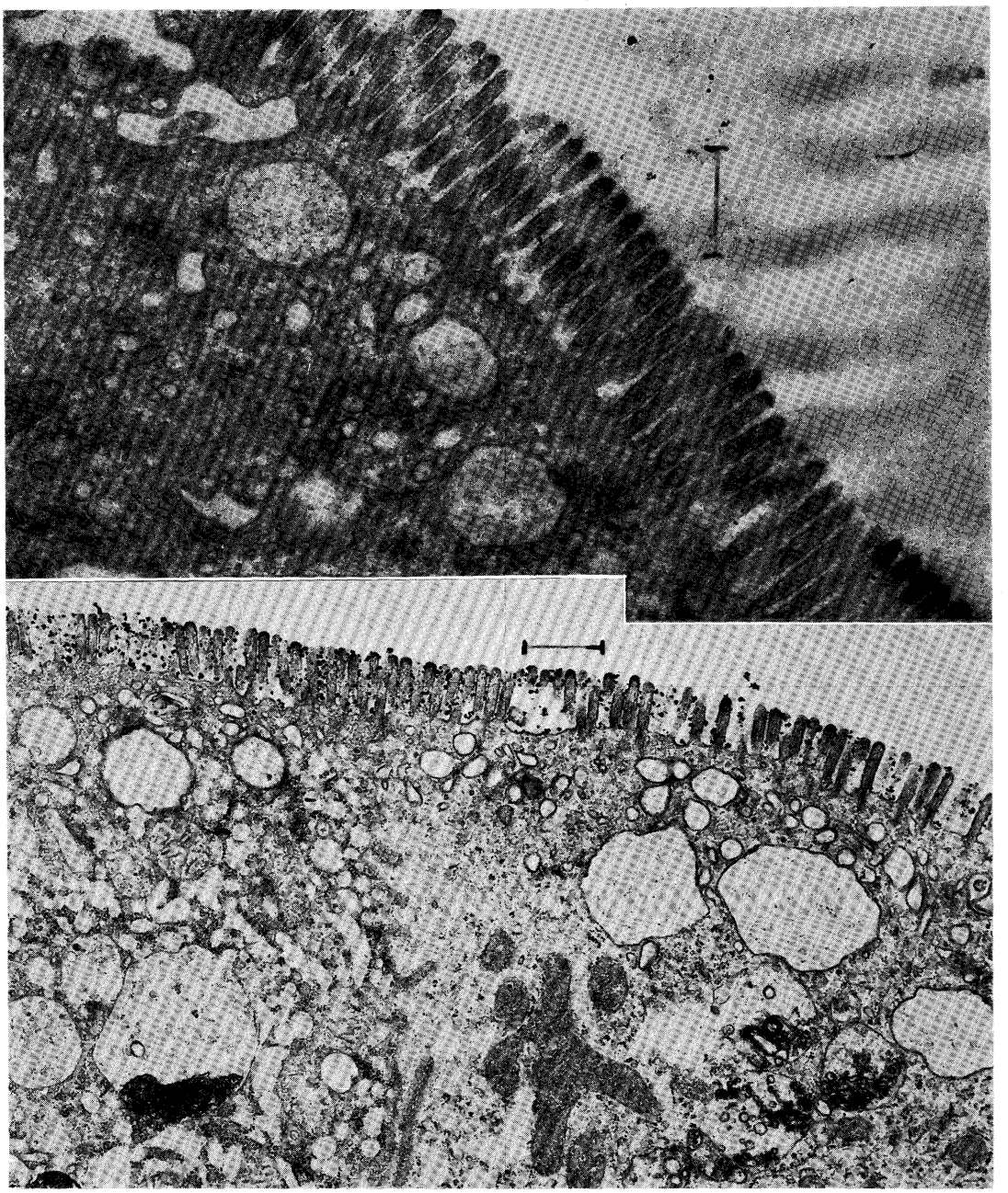


Photo. 15 (upper), Photo. 16 (lower).
Alkaline phosphatase activity in the intestinal absorptive cell from infantile, 7-day-old, mice. Activity of the enzyme is not proved in photo.15, and poorly observed in photo.16. x16000, x11000

Absorption test

1. Absorption of ferritin

It was noticed that a different distribution of ferritin which was injected directly into the intestine in the infantile group from that of the adolescent indicated a particular absorptive process of protein in the epithelium of the infantile mice.

The columnar absorptive cells of the infantile showed an existence of ferritin particles in different parts; numerous ferritin particles were found in pinocytotic indentation and vacuoles of the apical cytoplasm which were forming phagosomes (Photo. 17 and 18). In the deeper region of the cells, phagolysosomes were found instead of phagosomes, and ferritin particles reduced markedly (Photo. 17). There were few particles of which appearance were suspected to be ferritin outside of phagosome (Photo. 18). The basal region contained few particles of ferritin, but a few were scattered in the space between the epithelium and lamina propria mucosa (Photo. 19).

On the contrary, in the adolescent group, there were no ferritin particles in the apical region beneath the brush border. Even in case which contained exceptionally vacuoles, ferritin particles were not seen in the vacuoles. Particles of high density which were suspected to be ferritin under decomposition were found on the surface and inside of the enzyme layer(Photo.20).

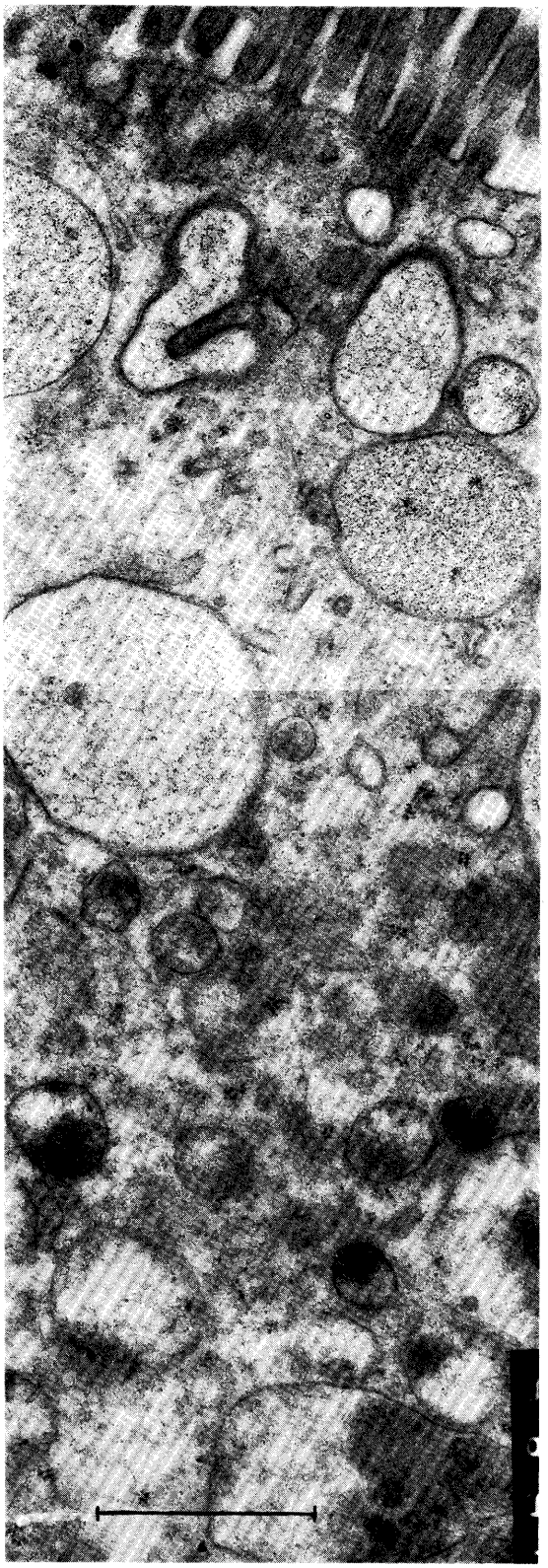
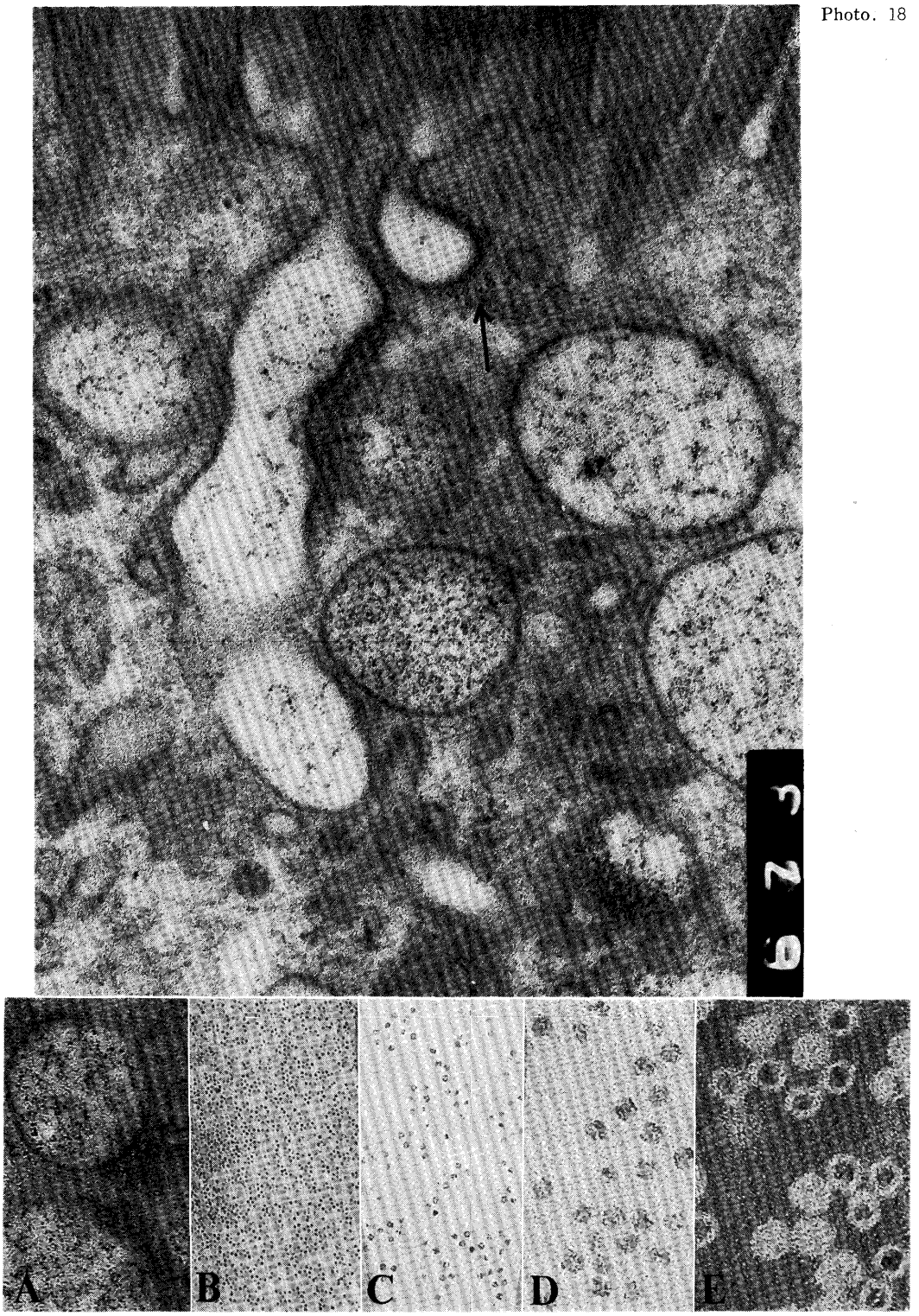


Photo. 17

Photo. 18



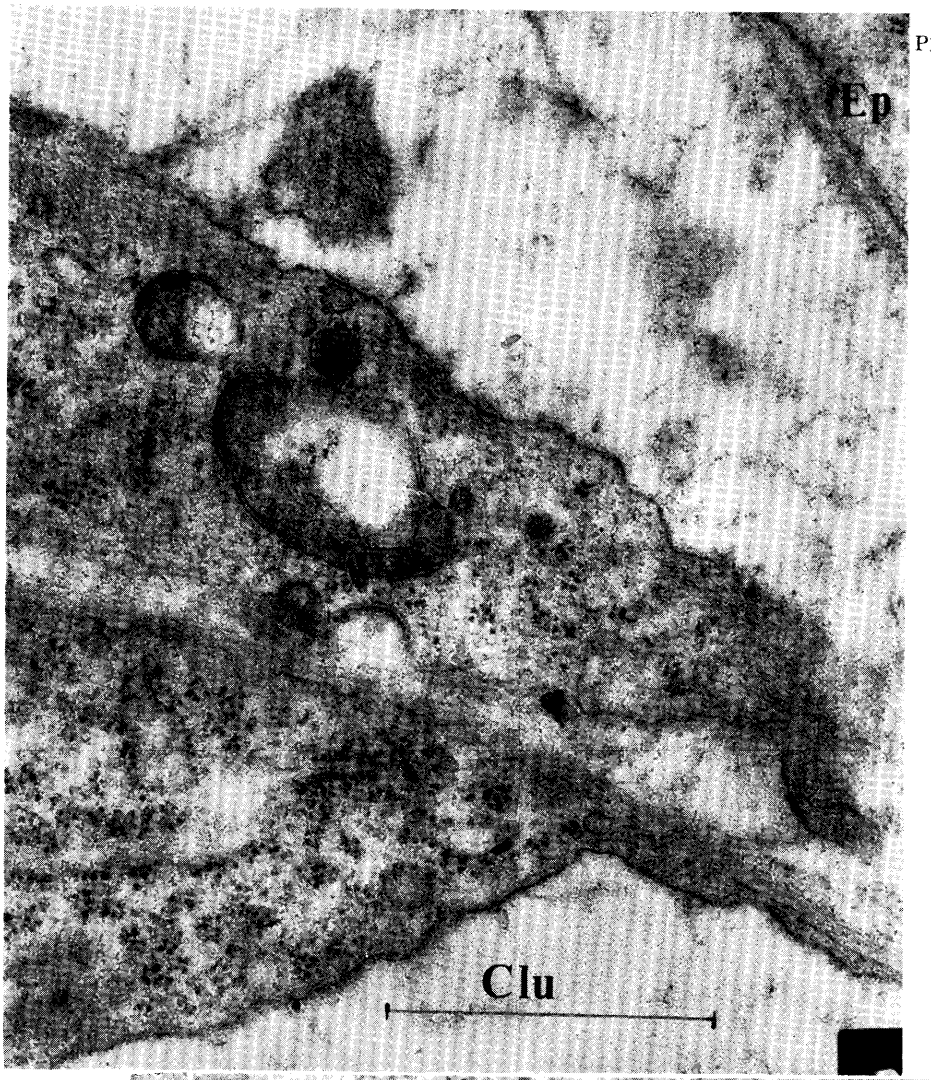


Photo. 19

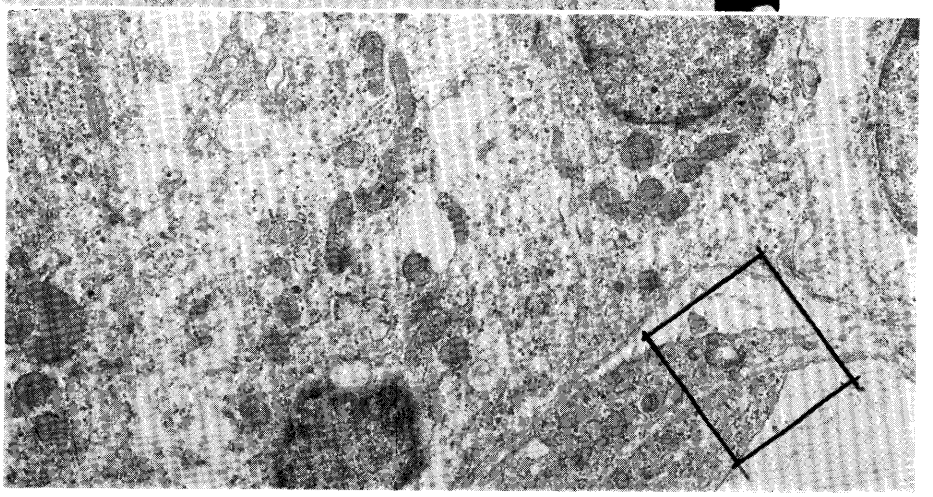
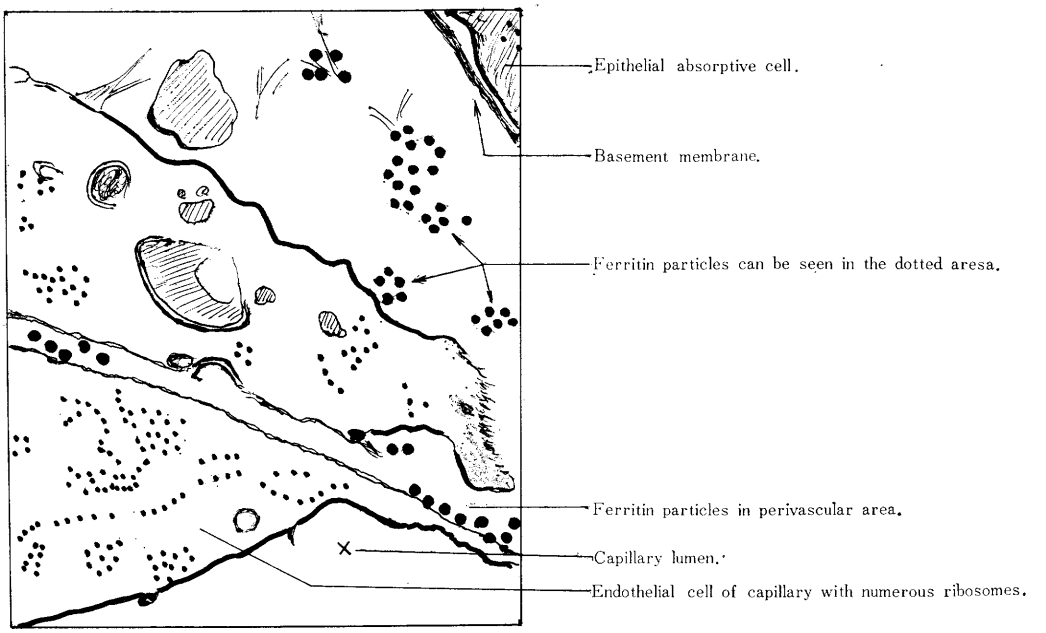


Photo. 19—A



Schema of Photo. 19

Photo. 17 : Upper part of the intestinal absorptive cell from infantile, 8-day-old, mouse. Numerous ferritin particles are found in the vacuoles (phagososomes) in the apical region. As they turn to phagolysosomes in the deper region, the number of ferritin particles in them makedly decreases. x29000

Photo. 18 : Apical region of the intestinal absorptive cell from infantile, 8-day-old, mouse. Ten per cent ferritin solution was directly injected into the intestine in ten minutes before fixation. A lot of ferritin particles are seen in pinocytotic indentation and vacuoles (phagosomes). It was confimed by serial section that the vacuoles were not connected to the pinocytotic indentation nor to the intestinal lumen. A few ferritin particles are also seen in the cytoplasm outside of the vacuoles (arrow). x66000.

The findings of ferrtin in various magnifications are shown under the photo.18.
A : ferritin in phagosome x66000, B : pure ferritin x66000, C : pure ferritin x150000
D : pure ferritin x500000, E : ferritin in negative staing x500000

Pnoto. 19 : Lamina propria mucosa of an intestinal villus from the same specimen with that of Photo. 18. Feritin particles are seen in the space between the epithelium and the capillary of lamina propria mucosa. The location of ferritin is indicated in the schema of the photograph. Photo. 19-A is a general view of the basal region of the epithelium. Photo.19 is a high magnificated picture of the bounded area in square. x44000

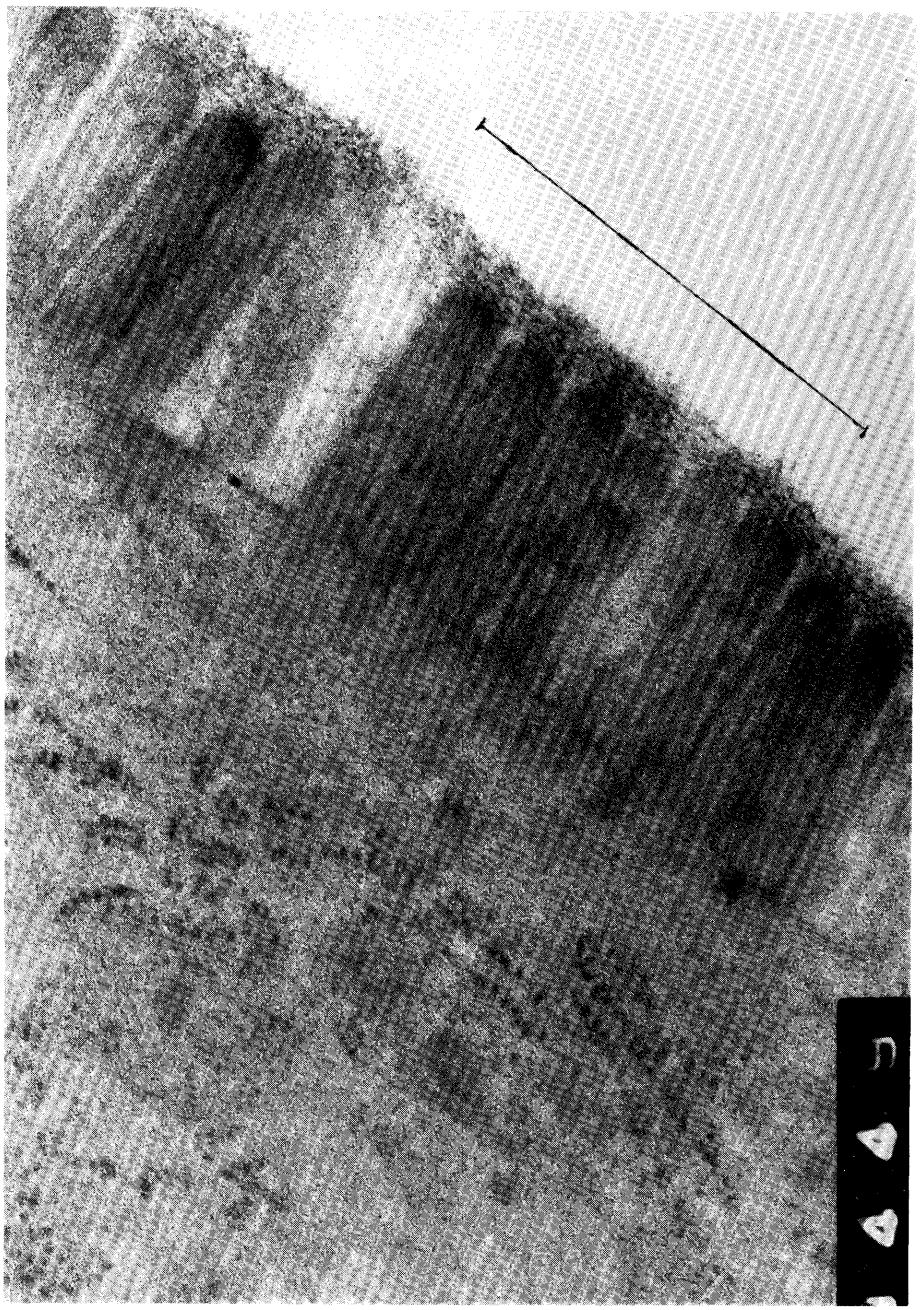
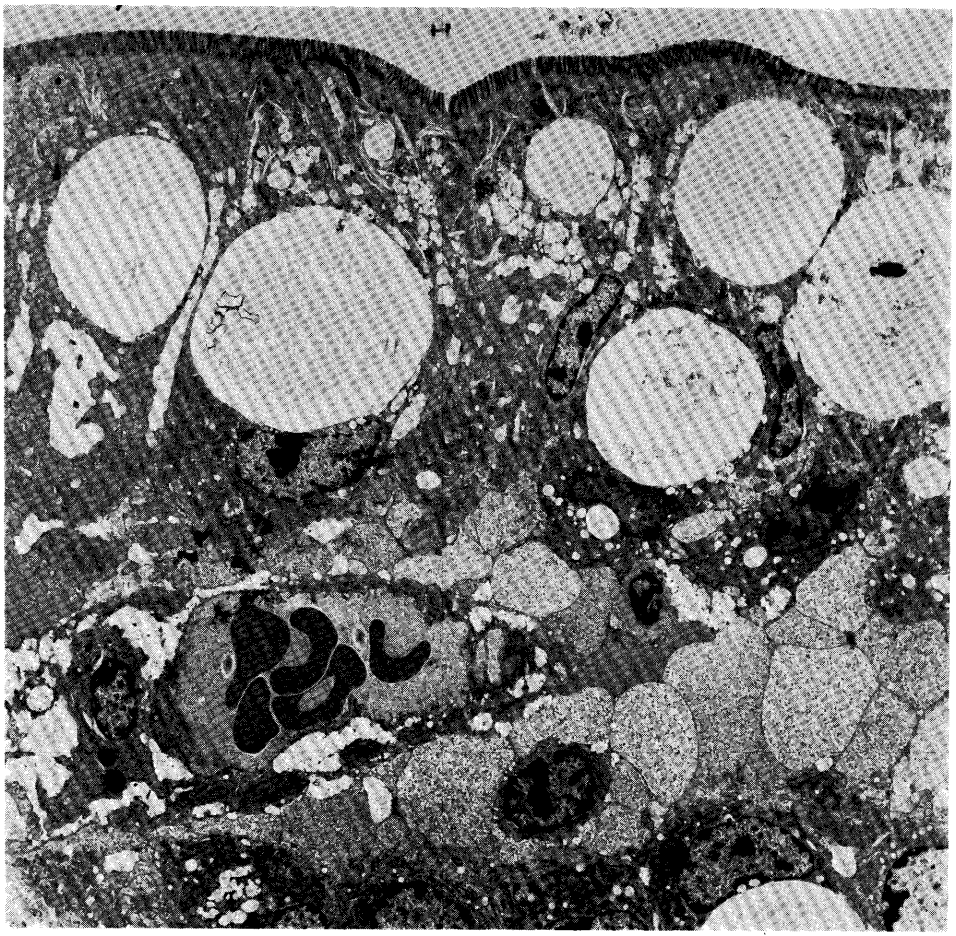


Photo. 20 : Apical region of a columnar absorptive cell of the intestinal epithelium from adolescent, 40-day-old, mouse. Ten per cent ferritin solution was directly injected into the intestine in ten minutes before fixation. Electron dense particles, presumably be ferritin, are mixed in the enzyme layer. No ferritin can be found in the cytoplasm. x76000

Photo. 21



2. Fat diet experiment in the adolescent group

In the feeding experiment of adolescent mice with fat diet, numerous fat droplets were proven in the epithelial absorptive cells and lamina propria mucosa under the light microscopy (Photo. 24). It is difficult to distinguish this findihg from that of the infantile mice fed with mother milk (Photo. 25).

According to the electron microscopic finding, in the infantile, there were a lot of vesicles and vacuoles in the apical cytoplasm (Photo. 1, 2 and 3). And in the middle part of the cell, especially in the supranucleal region, there were numerous phagolysosomes.

On the contrary, in the adolescent fed with fat diet, there were no vesicles nor vacuoles in the apical cytoplasm. But there were numerous vacuoles without limiting membrane in most part of cytoplasm other than apical region. Phagolysosomes were not observed. (Photo. 21 and 21-A, B, C and D)

Photo. 21－A

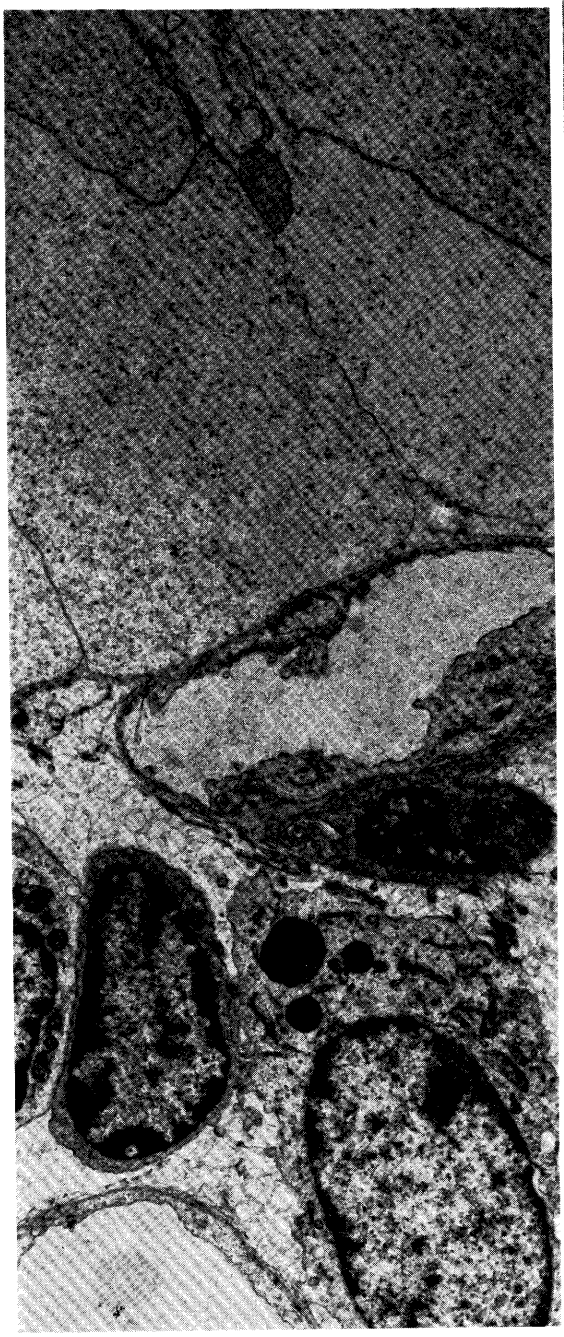


Photo. 21－B

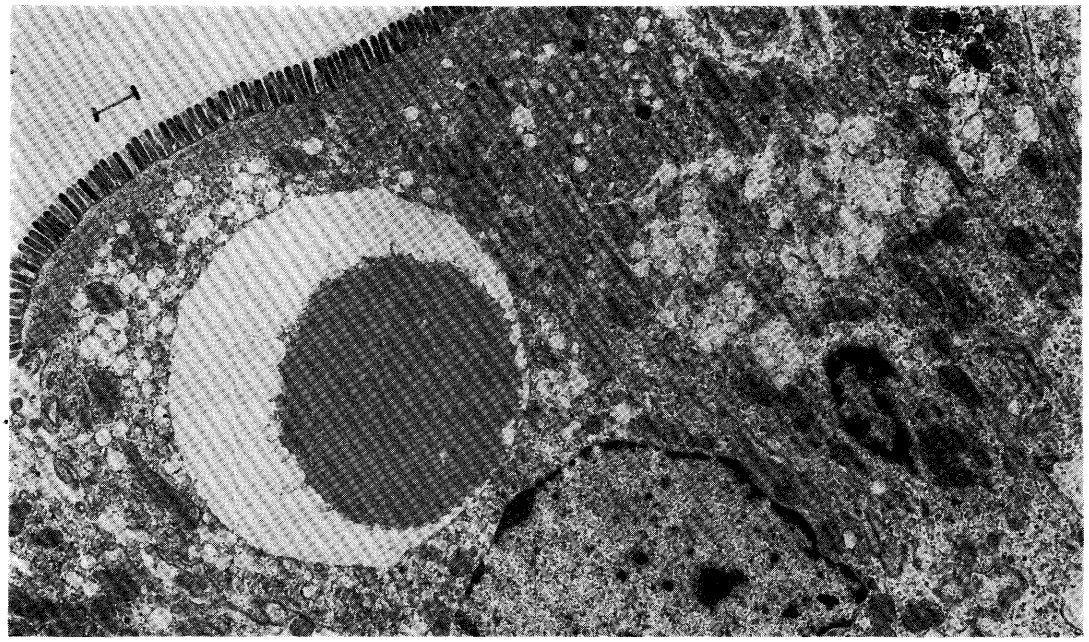


Photo. 21−C

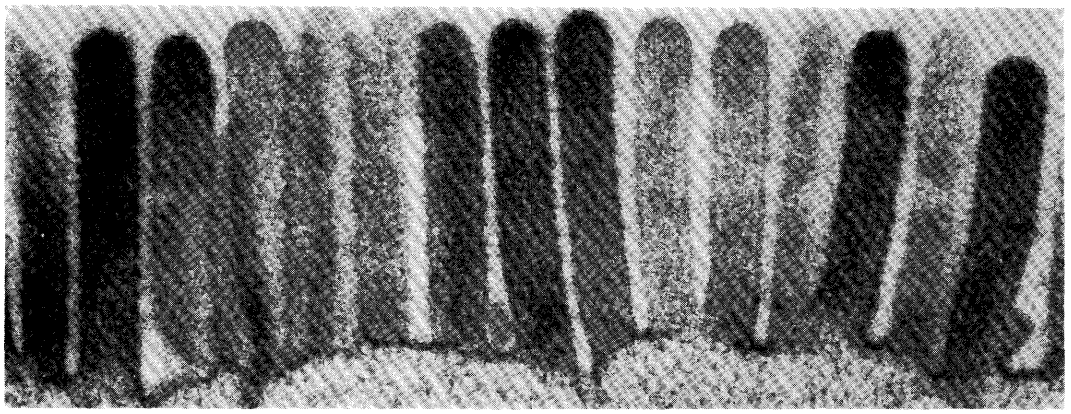


Photo. 21−D

Photo. 21 : General view of the epithelium and lamina propria mucosa of an intestinal villus from adolescent, 29-day-old, mouse that had been fed with fat diet for 24 hours. Cytoplasm of the absorptive cells are filled with vacuoles which are various in size. Some substance are found in them even after the treatment for the tissue by ethanol and aceton. Intercellular space is partially opend. Basal region of cytoplasm consists of homogeneous structure which seems to originate from fat. In the terminal web, however, no vacuoles nor pinocytotic indentations are seen (21-C). And the vacuoles are not bounded by a limiting membrane (21-B). 21-A showes basal region of the epithelial cells and lamina propria mucosa. Electron density of microvilli is homogeneously increased but trilaminal structure of the plasma membrane is clearly distinctive (21-D) x2400 (21-A x6600, 21-B x66000, 21-C x6000, 21-D x44000)

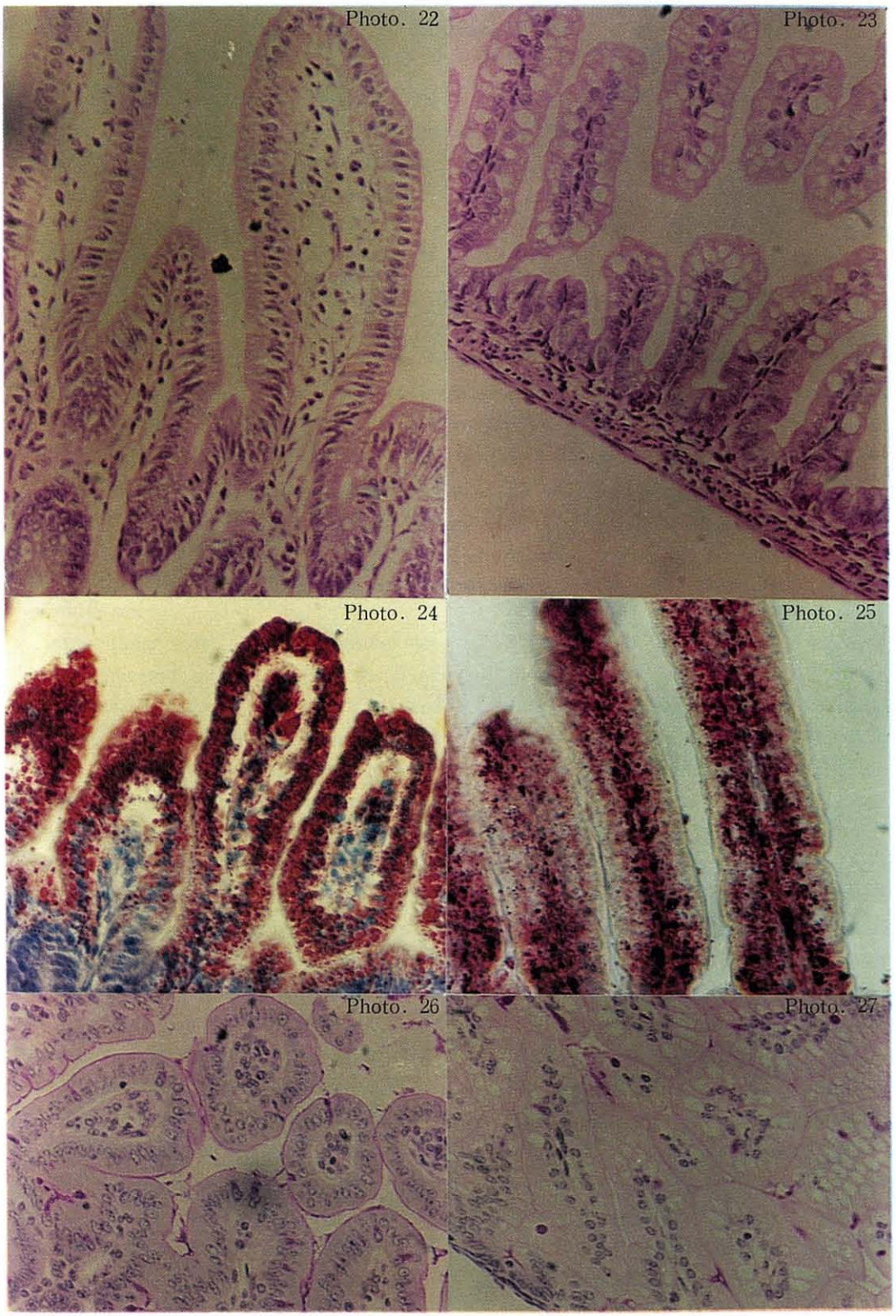


Photo. 22
Photo. 23
Photo. 24
Photo. 25
Photo. 26
Photo. 27

Photo. 22 : Intestinal villi of adolescent mouse. HE staining.
Absorptive cells are tall and thin, and vacuoles are not found in them.

Photo. 23 : Intestinal villi of infantile mouse. HE staining.
Absorptive cells are cubic, and vacuoles are seen in them.

Photo. 24 : Intestinal villi of adolescent mouse fed with high fat diet for 24 hours before fixation. Numerous fat droplets are seen. Oil Red O staining.

Photo. 25 : Intestinal villi of infantile mouse fed with mother's milk. Oil-Red-O staining.

Photo. 26 : Intestinal villi of adolescent mouse in PAS staining. The surface of the villi and goblet cells are markedly positive for the staining.

Photo. 27 : Intestinal villi of infantile mouse in PAS staining. The surface of the villi is negative for the staining, but goblet cells are positive.

Discussion

As mentioned in the introduction, the purpose of this study is to clarify the reason why the incidence of experimental cholera in mice with oral challenge of Vibrio cholerae is different according to the age of the animals, namely only infantile mice younger than 8 days old are susceptible to oral challenge with Vibrio cholerae. The result of the study will be discussed by item to item.

Enzyme layer[4]: It was noticed that the surface of the mucosa in the infantile mice was lacking in enzyme layer. This fact means that the ability for selection and digestion for absorption by means of various enzymes is almost defected in infantile mice.

Intercellular space : Opened intercellular space indicated one of the uncompleted structures of villus in the infantile. There is a hypothesis that the opened intercellular space may play an important role to exchange fluid from the lumen of the intestine to the body, and vice versa. So far, no distinct evidence was found to prove this, however, it can be presumed that the space may fascilitate an exchange of fluid under particular condition.

Vacuoles and pinocytotic indentation : Vacuoles and pinocytotic indentation in the absorptive cells of the infantile mean that the epithelial cells can ingest, by pinocytosis, various kinds of substance as foreign body including fat and protein.

Endoplasmic reticulum : There were a lot of smooth endoplasmic reticulums in infantile mice and on the other hand mostly granular endoplasmic reticulum in adolescent mice. It can be understood that the former should be related to lipid metabolism and the latter to synthesis of protein, which may play a significant role for producing enzymes in the brush border[5].

Lysosomes : The existence of numerous lysosomes in the absorptive cells of infantile mice also supports the above mentioned presumption. The process of the intracellular digestion can be comprehended by the fact that the ultra-structure of the absorptive cell is consisting of three different parts, the upper part with phagosomes, the middle with phagolysosomes and the basal with the assimilated substance.

Microvilli : Microvilli are the apparatus for the first step of absorption. It is supposed that large number of microvilli of the absorptive cell should play an active function for absorption. The structure of microvilli, in the adolescent, which have distinct trilaminal structure of the plasma membrane should be so tenacious as durable for active movement. However, the appearance of microvilli in infantile mice seemed to be fragile ; the trilaminal structure of the plasma membrane is lacking or poorly provided, and the surface of the membrane is rather smooth.

Polysaccharides[6],[7],[8] : It has been known that polysaccharides are functionally important substances in the cell membrane. The existence of polysaccharides in adolescent was distinctly proven by PAS and alcian blue staining. However, it was not recognized in infantile mice. This fact should be an important clue to understand a particular absorptive abnormality in infantile mice.

Alkaline phosphatase[3),9),10),11)] : Prominent activity of alkaline phosphatase was proven in the brush border of adolescent mice, and it was not proven in the infantile. It is supposed that various kinds of enzyme other than alkaline phosphatase may exist on the surface of the epithelium in adolescent mice, but is lacking in the infantile.

Absorption of ferritin[12),13),14)]: In the gastrointestinal tract, protein should be decomposed to peptide or amino acid before absorption. Ferritin given in the intestine of the adolescent was not detected in the absorptive cells in ten minutes after giving. The existence of enzyme layer with some electron dense particles indicated that ferritin was being decomposed by some proteolytic enzymes. In the infantile, however, numerous ferritin particles were found in the absorptive cells and even in lamina propria mucosa. This abnormal absorption in the infantile should be applied in case with absorption of toxin or other high molecular substance which are produced by some pathogenic organism in the intestine.

Feeding experiment with high fat diet[12),15),16)]: In adolescent mice fed with high fat diet, the light microscopic finding of the intestinal villi was similar to that of the infantile ; numerous fat droplets were seen in the absorptive cells and lamina propria mucosa. On the contrary, the electron microscopic finding indicated a distinctly different finding of the absorptive cells between adolescent mice fed with high fat diet and infantile mice ; there were no vesicles in the apical cytoplasm, vacuoles were not bounded by a limiting membrane and no lysosomal action was recognized. These findings suggest that fat could not be absorbed unless it was decomposed to fatty acid and glycerin or monoglyceride.

The first step for the establishment of infection with Vibrio cholerae should be a multiplication of vibrios in lumen of the small intestine. Then, a certain toxin or toxin-like substance will be produced by vibrios, which will act on the intestinal mucosae, so that a large amount of fluid will be accumulated in lumen of the intestine. And it is supposed that an accumulation of fluid in the intestine may provide a beneficial condition for further multiplication of vibrios. Thus, a characteristic symptom of cholera will develope acceleratively. It is supposed that a similar condition will be provided in the experimental cholera in mice.

The above mentioned differences which have been investigated from the comparative study between infantile and adolescent mice should be related to responses of the intestinal mucosa against toxin or toxin-like substance produced by Vibrio cholerae [17),18),19)]. The morphological and functional incompleteness of the intestine seen in infantile mice will provide a particular condition for establishing the infection with Vibrio cholerae. On the other hand, in adolescent mice, the intestinal mucosa is considered to be resistant against toxin or toxin-like substance except special cases; an accumulation of fluid will not occur in lumen of the intestine and multiplication of vibrios will not be allowed.

So far, any satisfactory explanation to clarify the host factor on the multiplicity of clinical manifestation in human infection

with Vibrio cholerae has not been obtained. However, it can be presumed that there may be some common and homologous factors in human cholera with those shown in the experimental cholera of infantile mice. For instance, deficiency of digestive enzymes will promote[20] an establishment of human cholera or incomplete structure or any damage of microvilli will give rise to fusing of villi which is able to be a cause of diarrea[21].

It is believed that the analysis of the infancy which has been understood to be a host factor in the experimental cholera of mice has presented some suggestion on the consideration of host factor in human cholera.

Summary

In the experimental cholera of mice, the infancy was analysed as one of host factors for the pathogeny, comparing the morphological, functional and histochemical differences of the intestinal mucosa between infantile and adolescent mice.

Infantile mice showed characteristics in the ultrastructure of epithelial absorptive cell, and enzyme layer and polysaccharides in the brush border were lacking. Alkaline phosphatase activity was not recognized and a particular ferritin ingestion was noticed in the infantile mice.

It was believed that these facts suggested a clue to explain a particular response of the intestinal mucosa of infantile mice against toxin or toxin-like substance produced by Vibrio cholerae in lumen of the intestine.

Acknowledgement

This study was carried out under the guaidance of Prof. K. Kobari, and the author wishes to express his sincerest gratefulness to him. The author's thanks is also offered to Dr. S. Takebayashi for his kind advice on electron microscopy, and to Miss Y. Kunita for her technical cooperation in providing the specimens for light microscopy.

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実験コレラ発症に関連する幼若マウス腸管の特徴

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摘　　要

感染症において，病原体が病因として決定的な役割を演ずるのは当然であるが，同時に宿主側の条件が発症機序に大きく関与する．コレラについても例外ではなく，感染個体の発症如何は宿主生体側の条件に左右されるとが大きい．氏家らが開発した幼若マウスにおける実験コレラでは，コレラ菌経口感染による発症率が，生後日数と確実に相関する．すなわち，生後１週間頃を境として発症率が著明に異なり，生後１ケ月以後のものでは全く発症が認められない．この実験コレラの成績に関しては，マウスの幼若性を発症のための宿主側条件の一つとして理解することが出来る．

実験コレラ発症要因としての幼若性を分析すれば，腸内細菌叢の未完成，消化酵素の欠如，腸内容の単純性さら

に免疫の異常などとともに腸粘膜細胞の構造ならびに機能の未熟さを挙げることが出きよう．この研究においては，腸粘膜の構造および機能を，生后1週間前後ならびに1カ月前後のマウスについて比較し，幼若性という特質を両者の相異からみいだそうと試みた．

幼若マウスに認められた特徴は次のようであった．

1，絨毛上皮の表面は enzyme layer を欠いている．

2，絨毛上皮層の細胞間隙が広く，細胞間の結合が弱い．

3，細胞質内には大小多数の空胞，液胞あるいは Phagosome を有し，同時に多数の pinocytotic identation を認める．

4．細胞質内には極めて多数の Phagolysosome が存在する．

5．Microvilli においては，限界膜の三層構造が不明瞭である．

6．刷子縁における多糖類の存在およびアルカリフォスファターゼの活性は極めて乏しい．

7．上皮細胞は蛋白質（フェリチン）を未消化（原形）のままとり込む．

以上のように，幼若マウスが示した腸膜粘の構造ならびに機能上の特異性は，腸管内においてコレラ菌が増殖した場合，その毒素または毒素様物質が容易に吸収される可能性を示している．

ヒトにおけるコレラ発症の生体側要因は今日全く不明であるが，ここに幼若性を分析して得たいくつかの条件の個々あるいはそのいくつかが重さなり合って，ヒトのコレラ発症に相似の条件を呈供することもありうると思われる．