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Effects of aging on the microclimate pH of the rat jejunum

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Abstract

The acidic microclimate layer in the vicinity of the cell surface of mammalian jejunum is important for absorption of some nutrients, such as small peptides and folate. The present study was undertaken to investigate the effect of aging on the cell surface pH (microclimate pH) of the jejunum of rats. The microclimate pH was measured in vitro in superfused preparations using single-barreled pH-sensitive microelectrodes filled with a liquid ion exchanger. The thickness of the microclimate layer was estimated by reading the distance of microelectrode advancements. The existence of a microclimate pH in the jejunum was confirmed in the senescent rats, but the value of the microclimate pH was significantly higher in the senescent (24 mo) rats (6.52 ± 0.02) than in the young-adult (6 mo) rats (6.09 ± 0.01) ($P < 0.01$). Na^+ removal from the perfusate or the addition of amiloride elevated the pH in the senescent rats as well as in the young-adult rats. The microclimate layer was slightly thinner in the senescent rats than in the young-adult rats. The acidity of the microclimate layer of intestinal surface is lower in senescent animals than in young-adult ones. One of reasons for this is the thinner mucus layer in senescent animals.

Keywords: Aging; Microclimate pH; pH; Sodium ion/proton antiporter; Unstirred water layer; Water layer, unstirred; (Rat jejunum)

1. Introduction

The functions and the structure of the gastrointestinal tract are affected by aging as in other visceral organs. Functional gastrointestinal disorders are common in the elderly. According to a nutrition survey, malnutrition was found in 6% of men and 5% of women between 70 and 80 years of age [28]. However, little is known about the pathophysiology of these problems. There are many possible causes of malnutrition, including behavioral factors such as decreased appetite, but some pathophysiological changes in absorptive systems may be responsible for this, since decreased absorption rates of glucose [38], amino acid [24], cholesterol [11], and calcium [2,5,15] with aging have been demonstrated.

One of the mechanisms that are responsible for the decrement in nutrients absorption would be age-related changes in the unstirred water layer (UWL). The importance of the UWL on the luminal surface of the enterocytes has been emphasized [11,12,38]. Hogben et al. [10] hypothesized that an acidic environment was present near the luminal surface of the small intestine to explain the unpredictable absorption of weak acids and bases from the luminal pH and the pH-partition theory. Lucas et al. [19] demonstrated the presence of an acidic microclimate pH in the vicinity of the cell surface of rat jejunum in vitro. Subsequently, Lucas' group [19] and other investigators [7,11,34] disclosed important properties of this pH layer. Owing to such an acidic pH in the vicinity of the surface of the intestinal epithelium and regulation of intracellular pH at a slightly alkaline level [35,36], a sufficiently large electrochemical potential gradient of H^+ is normally maintained across the brush-border membrane. Recent studies have provided strong evidence for the involvement of the microclimate pH in absorptive processes of certain nutrients and drugs [9,16,23,29–31,33]. Several H^+ gradient-driven transport systems have been shown to exist in the brush-border membrane: e.g., the peptide/H^+ cotransporter [9,16], the folate/OH^- antiporter [29–31], the guanidine/H^+ antiporter [23], and the β-alanine/H^+ cotransporter [39]. Thus, the physiological role of microclimate pH in absorption and secretion is evident. The impairment of formation of the microclimate pH as seen in patients with celiac sprue and Crohn's disease, is known to

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be associated with malabsorption of peptides and folate [1,21]. However, there have been no studies regarding the effects of aging on the microclimate pH. The present experiments were undertaken to investigate the effect of aging on the microclimate pH of the rat jejunum.

2. Materials and methods

2.1. Animals

Male Wistar rats were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). Rats were maintained at the Animal Experiments Center of the Hamamatsu University School of Medicine. Rats of two age groups were used in this study: a 6 mo rats group (representing sexually mature young adults beyond the stage of rapid linear growth), and a 24 mo rats group (representing senescent adults). In our center, the mean mortality until ~24 month is 50%. Rats were maintained on standard chow and were allowed to eat and drink tap water ad libitum. The photoperiod was 12:12 h light-dark from 07:00 to 19:00; room temperature was kept at 21–24°C.

2.2. Chemicals

Hydrogen ion ionophore II was obtained from Fluka Chemika (Buchs, Switzerland). Dimethyldichlorosilane was obtained from Serva Feinbiochemica (New York, NY, USA). Amiloride, dibutyryl cAMP, dibutyryl cGMP, DL-dithiothreitol (DTT) were from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and commercially available.

2.3. Tissue preparation and equipment

Animals (weight about 250–450 g) were anesthetized with urethane (1 g/kg body weight, intraperitoneal). The abdominal cavity was opened by a midline incision and a segment of the proximal jejunum, about 1 cm in length, just distal to the ligament of Treitz was excised after tying the borders with cotton threads. The segment was opened by a longitudinal incision along the antimesenteric border. After rinsing in physiological saline, the preparation was fixed in a small Lucite chamber with a gas-lift circulation system (Summit Medical, Tokyo, Japan). A part of the circulation system was dipped in a thermoregulator. The chamber had a base plate on which the tissue specimen was fixed with pins with luminal side upward. The tissue specimens were perfused with the standard solution or a test solution saturated with 100% O_2 at a rate of 10 ml/min. The temperature of the perfusion solution was kept at 37°C. All experiments were performed within 30 min after the excision. During this period of time, the tissue viability was well preserved as reported previously [36].

2.4. pH-sensitive microelectrode

The pH-sensitive microelectrode was manufactured accordingly to the methods described by Shimada and Hoshi [36]. Briefly, single-barreled microelectrodes (outer diameter = 1.1 mm) were made from fiber-built-in glass capillaries (Narishige, Tokyo, Japan). The glass capillaries were thoroughly washed with distilled water, then they were placed in a 20% solution of neutral detergent (v/v of boiled water) for 30 min. Subsequently, they were soaked in concentrated sulfuric acid for 30 min. After washing, the capillaries were placed in distilled water and warmed up until convection. They were then placed in an oven at 150°C for 1 h and kept in a desiccated chamber until use. The clean glass capillaries were pulled by using a vertical puller for manufacturing patch clamp pipettes (PB-7, Narishige) to obtain pipettes with a tip diameter of around 3–5 μm. The inner surface of capillary was silanized by exposing it to dimethyldichlorosilane vapor for 60–90 s. The silanized capillaries were baked at 200°C for 2.5 h. After cooling for a while, the tip was filled by injecting a small amount of H^+ ion sensitive resin from the back with a syringe. The composition of the H^+ ion sensitive resin (Hydrogen ion ionophore II) was 6% 4-nonadecylpyridine, 93% 2-nitrophenyloctylether, 1% potassium tetrakis(4-chlorophenyl)borate [6]. The shank of the glass electrodes was filled with a buffer solution (15 mM NaCl, 40 mM KH_2PO_4, and 23 mM NaOH). The resistance of the electrode was $(3–10) \cdot 10^8$ Ω, and the response time was less than 12 s.

2.5. Measurements of the microclimate pH profile

Due to the high resistance of the pH microelectrode, pH measurements were made in an earthed metal cage. A well-shielded wire was employed for the input circuit, and all pieces of the equipment, including the micromanipulators and stereoscopic microscope, were grounded.

As an indifferent electrode, a conventional microelectrode (whose tip diameter was less than 1 μm) filled with 3 M KCl was used. An Ag-AgCl wire in contact with 3 M KCl inside the electrode was connected to the input of an electrometer (FD-223, W-P Instruments, New Haven, MA, USA).

The pH values were calibrated at 37°C using 150 mM NaCl, 2 mM Hepes with various pH ranging 5.5–7.5. The pH of the solutions was adjusted by 2-(*N*-morpholino)ethanesulfonic acid (Mes). The average slope of the voltage-response of the pH-microelectrodes used in the present study was -58.5 ± 0.3 ($n = 128$) mV/pH in the above pH range. The electrode tip was advanced toward the intestinal surface with a micromanipulator. During this procedure, the voltage output of the electrode was continuously recorded. Before reaching the cell surface, a gradual acidification was usually recorded. Upon reaching the cell surface, a negative deflection of voltage

was recorded. This deflection indicates the penetration of the electrode tip into a cell. In this experiment, the lowest pH recorded just before the penetration into a cell was taken as the value of the microclimate pH or the 'cell surface pH'.

Usually, the pH-microelectrode was positioned at an angle of 60 degrees against the tissue surface. The excised tissues were perfused with the standard solution for 5 min before the control pH was measured at several points in one tissue specimen. And were perfused with a solution that contained one of the test substances for 15 min before measuring the pH.

2.6. Thickness of the microclimate layer

The thickness of the acid microclimate layer was estimated by reading the micrometer installed in a micromanipulator during advancement of the pH-sensitive electrode towards the surface of the intestine [37]. Usually, the electrode was advanced stepwise at a distance of 10 μm. From the number of steps from the minimum to the maximum pH changes and the angle of the electrode axis against the tissue surface, the thickness of the microclimate layer was estimated [34].

2.7. Perfusion solution

The standard perfusion solution used in the present study contained 145 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Hepes (pH 7.40 adjusted by Tris). NaCl was replaced by equimolar choline chloride to obtain the Na^+-free solution. When nutrients or drugs were added to the standard solution, its osmolarity was adjusted by reducing the content of NaCl.

2.8. Statistical analysis

Results are given as the mean ± S.E., n indicates the number of rats. In each case, the distribution of data was normal and, for this reason, significance levels were determined by Student's t-test [20], and were checked using the Mann-Whitney non-parametric significance test, but these data are not presented.

3. Results

3.1. Effects of aging on the microclimate pH

We first examined whether the acidic microclimate pH existed in the jejunum of the senescent rats. It was usual finding in the senescent rats as seen in the young-adult rats that pH value decreased when the pH-sensitive electrode was advanced from a certain point in the vicinity of the cell surface toward the cell membrane. Such a microclimate pH was stable at least for 30 min from the start of measurement (Fig. 1). The cell surface pH (the lowest microclimate pH) at 5 min after the start of experiments was higher (less acid) in the senescent rats (6.52 ± 0.02 (n = 60)) than in the young-adult rats (6.09 ± 0.01 (n = 60)) ($P < 0.01$) (Fig. 2). There was no significant difference in the pH between the villus tip region (6.53 ± 0.03) and the crypt region (6.49 ± 0.03) (n = 10) in the senescent rats. Similar observations were performed on the cell surface pH of the young-adult rats as found in the young-adult rats (6.11 ± 0.03 and 6.12 ± 0.04 (n = 4), respectively). In all following experiments, the pH measurements were perfused in the villus tip region.

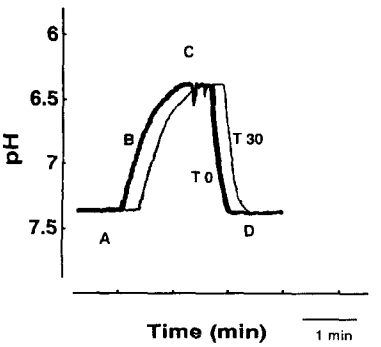

Fig. 1. A typical trace of the cell surface pH (microclimate pH) of the proximal jejunum in the senescent rats. The pH electrode was first placed in the bulk of the perfusion solution (A), then it was gradually advanced towards the cell surface (B). After reaching the maximum pH change (acidic pH), an abrupt negative deflection was usually recorded (C), after pulling back the pH-electrode, the pH returned to the original level (D). Two traces are superposed. One is that obtained immediately after the start of experiments (T0), the other was recorded after 30 min (T30). Similar observations were made in the young-adult rats.

3.2. Effects of Na^+-replacement and amiloride on the microclimate pH

Total replacement of Na^+ of the perfusion solution resulted in alkalinization of the cell surface pH in both

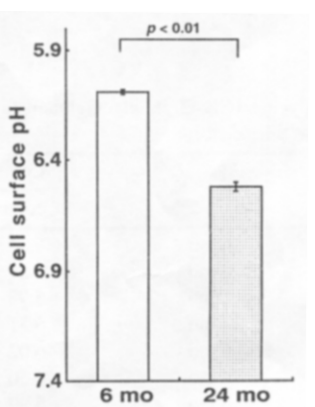

Fig. 2. A comparison of mean values of the cell surface pH of the proximal jejunum between two age groups (6 mo and 24 mo). pH values were determined after 5 min perfusion with the standard solution. Results are presented as the mean ± S.E. of 60 experiments.

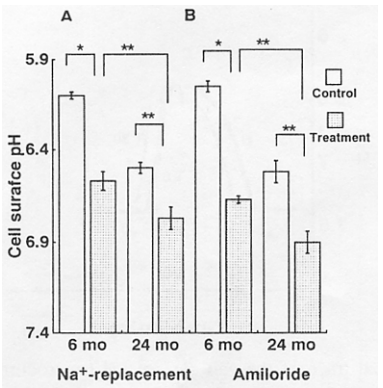

Fig. 3. Effects of Na^+-replacement and 1 mM amiloride on the cell surface pH of the proximal jejunum in the young-adult and the senescent rats. After 5 min perfusion with the standard solution, when the control measurements were carried out, the perfusion solution was changed to the Na^+-free solution (A) or a solution 1 mM amiloride containing (B), and the cell surface pH was recorded after 15 min. Results are presented as the mean ± S.E. of 10 separate experiments. Statistical significance: * $P < 0.01$, ** $P < 0.05$.

groups of animals (Fig. 3). In the senescent rats, it was 6.50 ± 0.03 in the presence of, and 6.77 ± 0.06 in the absence of Na^+ ($n = 10$). While in the young-adult rats, it was 6.14 ± 0.02 and 6.57 ± 0.05 ($n = 10$), respectively. The differences between the two conditions are statistically significant ($P < 0.05$).

The cell surface pH was also significantly alkalinized when 1 mM amiloride, a specific inhibitor of the Na^+/H^+ antiporter was applied to the perfusion solution in both age groups. In the senescent rats, the cell surface pH was 6.52 ± 0.06 in the absence of, and 6.90 ± 0.06 in the presence of amiloride ($n = 10$). In the young-adult rats, it was 6.05 ± 0.03 and 6.67 ± 0.02 ($n = 10$), respectively (Fig. 3). The differences between control and experimental conditions are also statistically significant.

3.3. Effects of glucose and other nutrients on the microclimate pH

Addition of 10 mM D-glucose or 10 mM D-fructose resulted in a significant acidification of the cell surface pH in both age groups. For examples, 10 mM D-glucose lowered the pH about 0.5 pH unit in both age groups. On the other hand, 3-*O*-methylglucose, lactose and galactose at the same concentration did not induce any significant change of the cell surface pH (Table 1).

Table 1
Effects of various sugars on the cell surface pH of the proximal jejunum from the young-adult and the senescent rats

Sugars (10 mM)	Age	Cell surface pH		*n*	Student's *t*-test
		control	treatment		
D-Glucose	6 mo	6.04 ± 0.01	5.52 ± 0.01	12	$P < 0.01$
	24 mo	6.57 ± 0.01	6.01 ± 0.03	12	$P < 0.01$
D-Fructose	6 mo	6.03 ± 0.05	5.66 ± 0.07	6	$P < 0.05$
	24 mo	6.50 ± 0.08	6.15 ± 0.09	6	$P < 0.05$
Lactose	6 mo	6.11 ± 0.05	6.09 ± 0.10	4	N.S.
	24 mo	6.53 ± 0.09	6.46 ± 0.15	4	N.S.
Galactose	6 mo	6.13 ± 0.07	6.07 ± 0.13	4	N.S.
	24 mo	6.60 ± 0.10	6.67 ± 0.15	4	N.S.
3-*O*-methylglucose	6 mo	6.01 ± 0.08	5.98 ± 0.12	4	N.S.
	24 mo	6.55 ± 0.12	6.54 ± 0.15	4	N.S.

The control values were determined after 5 min perfusion with the standard solution in this series. The values of experimental conditions were determined 15 min after the start of perfusion with a solution containing each sugar. Results are presented as the mean ± S.E.

Table 2
Effects of glycylglycine (Gly-Gly), glycylglutamate (Gly-Glu), L-alanine and L-glycine on the cell surface pH of the proximal jejunum from the young-adult and the senescent rats

Nutrients (10 mM)	Age	Cell surface pH		*n*	Student's *t*-test
		control	treatment		
Gly-Gly	6 mo	6.01 ± 0.03	6.29 ± 0.04	8	$P < 0.01$
	24 mo	6.56 ± 0.03	6.72 ± 0.05	8	$P < 0.01$
Gly-Glu	6 mo	5.98 ± 0.03	6.20 ± 0.03	4	$P < 0.01$
	24 mo	6.51 ± 0.04	6.70 ± 0.07	4	$P < 0.05$
L-Alanine	6 mo	6.02 ± 0.02	6.14 ± 0.02	6	$P < 0.05$
	24 mo	6.50 ± 0.06	6.35 ± 0.07	6	N.S.
L-Glycine	6 mo	5.99 ± 0.09	6.05 ± 0.12	4	N.S.
	24 mo	6.45 ± 0.10	6.52 ± 0.14	4	N.S.

The control values were determined after 5 min perfusion with the standard solution. The values of experimental conditions were determined 15 min after the start of perfusion with a solution containing a test substance. Results are presented as the mean ± S.E.

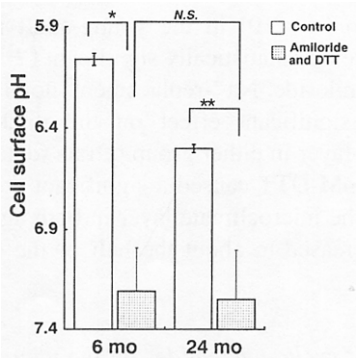

Fig. 4. Effect of 1 mM DTT on the cell surface pH of the proximal jejunum in the young-adult and the senescent rats. Control values were obtained after 5 min perfusion with the standard solution. Then the perfusion solution was changed to the 1 mM DTT containing solution, and the cell surface pH was recorded after 15 min. Results are presented as the mean ± S.E. of 8 separate experiments. Statistical significance: * $P < 0.01$, ** $P < 0.05$.

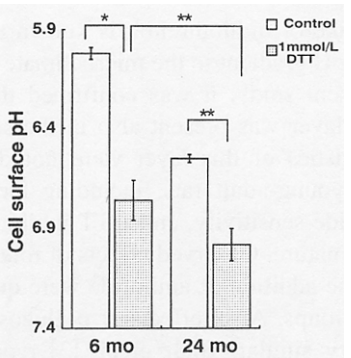

Fig. 5. Effect of additive treatment of 1 mM amiloride and 1 mM DTT on the cell surface pH in the young-adult and the senescent rats. After 5 min perfusion with the standard solution, the tissues were perfused with a solution containing 1 mM amiloride and 1 mM DTT. The cell surface pH was recorded after 15 min. Results are presented as the mean ± S.E. of 4 separate experiments. Statistical significance: * $P < 0.01$, ** $P < 0.05$. N.S.: means not significant.

As shown in Table 2, 10 mM glycylglycine and 10 mM glycylglutamate caused a significant alkalinization of the cell surface pH in both groups. Glycylglycine elevated the pH by about 0.28 in the young-adult rats, while it causes slightly weak alkalinization in the senescent (by about 0.16 pH units). 10 mM L-glycine did not affect the cell surface pH significantly. L-Alanine had inconsistent effects, it slightly increased pH value in the young-adult rats and slightly acidified in the senescent rats.

3.4. Effect of dithiothreitol on the microclimate pH

When DL-dithiothreitol (DTT), a mucolytic agent, was added to the perfusion solution at 1 mM, the acidity of the microclimate layer was reduced significantly in both groups. In the senescent rats, the cell surface pH was 6.53 ± 0.04 in the absence of, and 6.76 ± 0.08 in the presence of DTT ($n = 8$); whereas in the young-adult rats, it was 6.11 ± 0.02 and 6.55 ± 0.05 ($n = 8$), respectively (Fig. 4).

Fig. 5 shows the additive effect of 1 mM amiloride and 1 mM DTT treatment on the cell surface pH. The acidic cell surface pH disappeared almost completely. Regarding this combined effect, no significant difference was observed between two age groups.

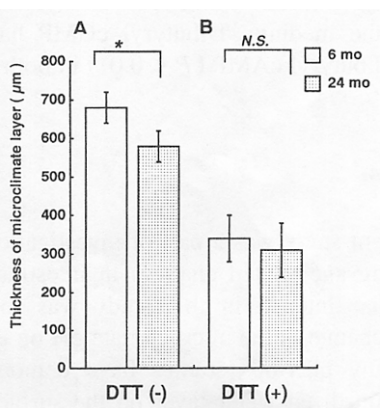

Fig. 6. Estimated values of the thickness of the acid microclimate layer in the proximal jejunum of the young-adult and the senescent rats. (A) Control values which were obtained after 5 min perfusion with the standard solution. After 3–20 min of perfusion, the thickness of the microclimate layer did not change significantly. (B) The thickness of the microclimate layer after 10 min perfusion with a solution containing 1 mM DTT. Results are presented as the mean ± S.E. of 4–6 separate experiments. Statistical significance: * $P < 0.05$. N.S.: means not significant.

3.5. Thickness of the microclimate layer

The thickness of the microclimate layer was estimated to be 580 ± 40 μm ($n = 10$) in the senescent rats and

Table 3
Effects of cyclic nucleotides on the cell surface pH of the proximal jejunum from the young-adult and the senescent rats

Cyclic nucleotides (2 mM)	Age	Cell surface pH		n	Student's t-test
		control	treatment		
Dibutyryl cGMP	6 mo	5.99 ± 0.05	6.29 ± 0.09	6	$P < 0.05$
	24 mo	6.40 ± 0.04	6.72 ± 0.11	6	$P < 0.05$
Dibutyryl cAMP	6 mo	6.04 ± 0.07	6.46 ± 0.13	5	$P < 0.05$
	24 mo	6.39 ± 0.05	6.65 ± 0.05	5	$P < 0.05$

The control values were determined after 5 min perfusion with the standard solution. The values of experimental conditions were 15 min after the start of perfusion with a solution containing the test substance. Results are presented as the mean ± S.E.

680 ± 40 μm ($n = 10$) in the young-adult rats (Fig. 6). The difference was statistically significant ($P < 0.05$). Neither 1 mM amiloride, Na^+-replacement, nor 10 mM D-glucose had a significant effect on the thickness of the microclimate layer in either group of rats (data not shown). However, 1 mM DTT caused a significant decrease of the thickness of the microclimate layer in both age groups, the thickness decreased to about the half of the control value (Fig. 6).

3.6. Effects of cyclic nucleotides on the microclimate pH

We examined the effects of cyclic nucleotides on the cell surface pH, since there have been reports suggesting an important role of them for intestinal H^+ secretion and other ion transport. As shown in Table 3, the cell surface pH became significantly alkaline in both age groups when 2 mM dibutyryl cGMP and 2 mM dibutyryl cAMP were applied to the medium. Dibutyryl cGMP had a greater effect than dibutyryl cAMP ($P < 0.01$) in both age groups.

4. Discussion

The present study was a part of investigations aimed at understanding age-related changes in intestinal functions. The particular interest in this study was to define the age-related change in the microclimate pH on the intestinal surface. Many previous studies have demonstrated that there is an unstirred water layer on the surface of the rat proximal jejunum where acidic pH is maintained not only in vivo [17] but also in vitro [7,17,19,27,34]. In addition to direct measurements using pH-sensitive electrodes, observations with a pH indicators (dyes) have also shown that the mucosal surface of the rat jejunum has an acidic pH [32]. Absorption of some nutrients and electrolytes are dependent on a proton gradient across the luminal membrane of enterocytes.

It was found that the microclimate pH was more alkaline in the senescent rats than in the young-adult rats. There are at least two important mechanisms involved in the maintenance of the normal intestinal acidic microclimate pH. The microclimate pH of jejunal surface is dependent on the dynamic equilibrium of H^+ secretion into the layer and diffusion out of the layer. The alkalinization of the cell surface pH due to elimination of Na^+ from the bathing medium or the presence of amiloride in the medium strongly suggests that the secretion of H^+ is mediated by the Na^+/H^+ antiport at the brush border membrane. The presence of the Na^+/H^+ antiporter is well documented for the brush border membrane of the enterocytes [18]. The second possible mechanism is the formation of a mucus layer, since intestinal mucin retards H^+ diffusion [26] and behave like an anion-exchanger [8]. Destruction or solubilization of mucus by a S-S reducing agent, such as *N*-acetyl-L-cysteine or dithiothreitol, is known to reduce significantly the pH gradient in the microclimate layer [17,34].

In the present study, it was confirmed that the acidic microclimate layer was present also in the senescent rats. The characteristics of this layer were not different from those in the young-adult rats, including the Na^+-dependence, amiloride-sensitivity, and DTT-inhibitable or nutrient-affectable nature. Observed effects of total replacement of Na^+ and the addition of amiloride were quite similar in both animal groups. Also, effects of D-glucose and D-fructose were very similar. Blair et al. [3] reported the enhanced production of hydrogen ions in the rat proximal jejunum when these sugars are present. On the other hand, lactose, galactose and 3-*O*-methylglucose had no effect. Glycylglycine and glycylglutamate which are known to be co-transported with H^+ induced an alkalinization, whereas, glycine, which is co-transported with Na^+ had no effect. These data clearly indicate that the microclimate pH layer is maintained in the senescent rats by the same mechanisms as in the young-adult rats.

Shiau et al. [32] showed that removal of the mucus layer in the rat jejunum by mild shearing force abolished the formation of an acidic microclimate. They concluded that mucus on the surface of the intestinal epithelium was the major factor to maintain a low-pH compartment. On the other hand, Forlong et al. [8] showed that the mucus obtained from the small intestine acted as an anion exchanger, this property seems to hold H^+ in the layer in contrast to gastric mucus which behaves like a cation exchanger.

A number of studies have reported [11,12,38] the difference in UWL thickness between young and old animals. Hollander and co-workers [11–13] concluded that absorption of fatty acids from micellar solutions as well as absorption of cholesterol and vitamin D increased with aging. They suggested that these findings could be explained by due to an increase in mucosal surface area per unit length of gut and a decrease in UWL thickness. In the present study, it was revealed that in the senescent rats, the acid microclimate layer was thinner and the cell surface pH was more alkaline than in the young-adult rats. Assuming that the chemical nature of mucus of the senescent rats is not different from that of the young-adult rats, a decrease of the mucus layer thickness would result in a reduced resistance to H^+ diffusion, and the diminished microclimate acidity. The changes in the activity of the Na^+/H^+ antiport should be taken into account for the diminished acidity of the microclimate layer. Further studies will be needed to determine the changes of the ability of the Na^+/H^+ antiport with age.

Recent studies have provided ample evidence for the involvement of the microclimate pH in the absorption processes of certain nutrients [9,29–31,33]. The age-related decrease of the microclimate acidity as observed in this study, suggests that changes of intestinal absorption may occur with age. It has been clearly established that

peptide transport across the brush-border membrane of the small intestine is H^+-driven [9]. There may be other processes in which the mucosal surface acidity or the H^+ gradient formed across the luminal membrane plays an important role. One such processes is absorption of calcium, which must be solubilized and ionized before being transported across the intestinal epithelium. Most of the calcium in food-stuff and drinks is in a hardly soluble or insoluble form. Gastric HCl secretion has been considered important for the solubilization and ionization of calcium salts in ingested foods [25]; however, Bo-Linn et al. [4] demonstrated that either large doses of cimetidine, which effectively inhibits gastric HCl secretion, or a neutralization of gastric content by a pH-stat method, had no effect on calcium absorption from $CaCO_3$. The intestinal solubilization and ionization of calcium salts at the microclimate layer is more important for calcium absorption (unpublished data). Studies in mammals have shown that intestinal calcium absorption declines with age [2,5,15]. Many studies have been undertaken to clarify the effect of aging on calcium absorption, but none of them paid an attention to the role of the intestinal surface mucus layer having acidic pH. It is quite probable that the decreased acidity of this layer in the senescence would explain, at least in part, the decrease of calcium absorption. The other important role of the microclimate pH is the lipid absorption. Shiau demonstrated that alteration of the microclimate pH has a significant effect on intestinal fatty acid uptake [35]. Low-pH compartment may play a key role in fat absorption. Although, studies of the intestinal lipid absorption in aged animals have yielded conflicting results [14], the raised surface pH values presented here would help to explain the alteration of lipid absorption with age.

The mucosal surface pH is controlled by intracellular cyclic nucleotides [22,36]. A simplified view of the intestinal acid-base movement and its control by the second messengers is that cGMP modulates the Na^+/H^+ antiport in the proximal jejunum and that cAMP modulates the anion exchanges. It is not clear that, the changes in the transporters and/or intracellular regulation of the transporter activities may undergo age-related changes. Accordingly, the effects of cAMP and cGMP were tested to find any differences in response between two age groups. In this study, both nucleotides changed the microclimate pH similarly in the senescent and the young-adult rats. These nucleotides did not change the thickness of the microclimate layer in both age groups (data not shown). Thus, any differences in the response were could not find. The age-related change in intracellular regulation of the transporter activity may be, if any, small; but further studies are needed for understanding of the age-related changes in intracellular mechanisms.

In summary, the results of the present study confirmed the existence of a microclimate pH in the senescent rats. Compared with the young-adult animals, the senescent rats had a higher (less acid) cell surface pH, and a thinner microclimate layer. A thinner mucus layer is suggested to be mainly responsible for a decrease in jejunal surface acidity in the senescent rats, but the possibility of a decreased activity of the Na^+/H^+ exchange could not be excluded.

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