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# Effect of Noise on the Morphology of the Intestinal Mucosa in Laboratory Rats

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**To determine whether noise-induced stress disrupts the intestinal mucosa of laboratory rats, one group of 8 rats ('noise' rats) was subjected to 15 min of white noise (90 dB) daily for 3 wk. Another group ('quiet' rats) was housed for 3 wk in an acoustically similar room but with no additional noise. A 3rd group ('recovery' rats) was housed in the noise room for 3 wk and then in the quiet room for a further 3 wk. The ilea were fixed for microscopy. Villi adjacent to Peyer patches showed significantly more degranulated mast cells (mean ± standard error of the mean, 3.95 ± 0.80 versus 0.35 ± 0.29, respectively) and eosinophils (mean ± standard error of the mean, 9.46 ± 0.44 versus 4.58 ± 0.38) per villus section in noise rats than in quiet rats. Similar results were obtained with rooms reversed, to account for any differences in room characteristics. The mean width of villus laminar propria was significantly greater in noise rats than quiet rats, suggesting edema. In addition, mucosal epithelial cells of noise rats were often separated, sometimes detaching from the basement membrane, whereas those of quiet rats were intact. Behaviorally, noise rats exhibited significantly more grooming and rearing than quiet rats. Compared with noise rats, recovery rats showed no reduction in mast cell degranulation or mean width of villus lamina propria, but there were increased numbers of secreting goblet cells in villi adjacent to Peyer patches and some recovery of epithelial integrity.**

**Abbreviations:** ANOVA, analysis of variance; CL, central lacteal; DAB, diaminobenzidine; DMC, degranulated mast cell; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; $H_2O_2$, hydrogen peroxide; ROS, reactive oxygen species

Mental stress has been shown to aggravate intestinal disease, such as Crohn disease and colitis, in humans,[11,12,18] but the effects of noise stress per se on intestinal structure have received little investigation. The intestinal epithelium provides a selective barrier to the transmucosal exchange of solutes and provides protection against potentially noxious agents that normally reside within the gut lumen. Any condition that disrupts this protective barrier allows inappropriate introduction of numerous antigens, microorganisms, and toxins into the lamina propria and ultimately the systemic circulation.

Mental stress also affects the physiologic responses of research animals.[10,27,29,35,36] In a previous study, we showed that environmental stress (that is, high personnel activity within animal housing facilities) promotes intestinal disorders in rats.[36] When rats were housed in a high-activity room for several weeks, the intestinal symptoms that they displayed were identical to those of inflammatory bowel disease. The intestinal mucosal epithelium was disrupted, a large proportion of mast cells in the intestinal mucosa and mesentery had degranulated, the number of fenestrae in mucosal capillary endothelium increased, and mesenteric microvessels were more permeable than those of rats housed in a low-activity room. Disruption of the intestinal epithelium reduces the barrier function of the intestine such that bacteria, fungi, parasites and their toxins, undigested protein, fat, and waste normally not absorbed into the bloodstream in the healthy state, pass through a damaged, hyperpermeable, 'leaky' gut and enter the bloodstream, particularly if there is also microvascular damage. These microbes and toxins, if present in large enough amounts, may overwhelm the liver's ability to detoxify.

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Excessive penetration of antigens through the epithelial layer also can result in inappropriate immune stimulation, leading to chronic gastrointestinal inflammation. Immune stimulation occurs in the intestine by means of Peyer patches, which are organized lymphoid tissues in the lamina propria of the small intestine where immune responses to antigens may be initiated.[24] In addition, some lymphocytes have homing receptors on their surface that preferentially promote adherence to the endothelium of microvessels in Peyer patches and subsequent emigration from the blood into them.[23] The basic cellular mechanisms responsible for the structural changes that occurred in response to high personnel activity in the animal facility remain obscure. It is possible that excess noise may have been a major factor contributing to the results.

Clough[10] cites studies showing that noise can have many different nonauditory effects on animals' physiology, such as hypertension, elevated levels of cholesterol, and increased atherosclerosis, reduction in body weight, and changes in immune response. Schmid and colleagues[33] demonstrated that noise caused increased concentrations of epinephrine in the blood of rats. Armario and coworkers[2] showed that exposure of rats to chronic noise caused decreases in blood glucose and insulin concentrations. Guha and colleagues[14] showed that exposure of rats to 80 dB noise for 1 or 2 h produced a marked decrease in gastric secretion and a concomitant increase in plasma corticosterone. When Van Raaij and coworkers[34] exposed rats to 90-dB noise for 540 min spread over 8 d, the animals showed an increased basal plasma corticosterone concentration. These increases in plasma concentrations of epinephrine and cortisone suggest that noise induces a stress response in rodents.

Although an increase in noise level can be an immediate and effective factor in producing a stress reaction in the body,[13] there has been very little study of the effects of environmental noise on intestinal diseases, either in animals or humans. Only

2 published studies so far have reported the effect of noise on the intestinal mucosa in laboratory animals. Bueno and Gue[8] reported that exposing mice for 20 min to music at ≤90 dB caused increased gastric emptying. Bijlsma and colleagues[6] showed that rats exposed to 95-dB noise pulses of 45 min hourly for 12 h daily for 8 d demonstrated increased flux of horseradish peroxidase across the isolated intestinal mucosal epithelium. Surprisingly, rats exposed to 105-dB noise did not show this effect, and it was hypothesized that this level of noise had damaged the rats' cochlear cilia.

We therefore designed experiments to test the hypothesis that noise was the leading factor contributing to the intestinal inflammation observed in rats housed in a facility with high personnel activity and that the noise was causing psychologic stress. In these experiments, 2 groups of rats were housed in separate but identical rooms with a noise generator in 1 of the rooms. The rats in the 'noise' room were exposed to 90-dB noise for 15 min daily starting at 18:00. This intensity of noise is similar to that encountered in some typical animal facilities. When observed several hours later at night (the animals' active period), the behaviors of both groups of rats were compared and assessed for signs of stress, such as excessive grooming, rearing, and yawning.[22,37] Initially, 'fighting' was selected as a stress parameter, but it was difficult to distinguish between real and 'play' fighting, and when combined, no significant difference between groups was observed. Therefore 'fighting' was not used as a parameter. After 3 wk, the animals were anesthetized and examined for damage to the intestinal mucosa. The experiments were repeated with the noise generator moved to the other room, so that the effects of the possible differences between the rooms could be eliminated. In addition, a 3rd group of rats was exposed to noise for 3 wk and then moved to the quiet room for several weeks, to determine the extent of recovery. If our hypothesis were correct, the rats exposed to daily noise would exhibit increased grooming, rearing, and yawning, and would show signs of intestinal inflammation, similar to the rats in the high-personnel-activity environment. Preliminary results of this study have been published in the form of an abstract.[4]

## Materials and Methods

**Experimental regimens and groups.** Male Sprague Dawley rats, weighing 300 to 350 g, were obtained from Harlan (Indianapolis, IN). Monthly serology, bacteriology, and parasitology evaluations are performed on animals from each virus-free barrier at Harlan. The rats were transported by truck to our animal facility, where monthly tests are performed on sentinel rats. On arrival, the animals were housed in identical accommodation but in 2 different rooms. The animal facility is small with low personnel activity, and 2 rooms (each 10 × 12 ft [3.0 × 3.7 m]) were chosen deliberately so as to be remote from noisy air vents, cage washers, etc. The temperature ranged between 22 and 23 °C, and the humidity was kept between 55% and 60%. In each room, 10 to 20 rats were housed 2 per cage (45 × 24 cm) containing hardwood chip bedding (Sanichip, Harlan) and were on similar diets (Tech Lab 485 Mouse and Rat Chow, Harlan) and light cycles (lights on, 6:00 to 18:00). No other rats, apart from those participating in this study, were housed with them. A technician entered both rooms once daily to feed and tend to the rats. Apart from the investigator, the technician was the only person to enter the rooms, and he was instructed to perform his duties at the same time each day. Both rooms had identical lighting arrangements of the same intensity, and each room was thoroughly disinfected before use to remove extraneous odors.

The 1 intentional difference between the environments in the 2 rooms was that the rats in 1 of the rooms received an additional stimulus each day at the same time every day (just before the lights were switched off at 18:00) for 3 wk; that is, white noise from an electronic noise generator (90-dB overall noise level) for 15 min. These rats are referred to as the 'noise' rats. The noise level of 90 dB (averaged over frequencies from 10 to 10,000 Hz) is similar to that produced by cage washers and air conditioners unless adequate care is taken, and it is relatively low compared to the 110-dB tolerance level of rats.[3,25,29] The rats in the other room did not receive the white-noise stimulus. These rats are referred to as the 'quiet' rats. The frequency distribution of the noise stimulus and the background noise in both rooms is shown in Figure 1.

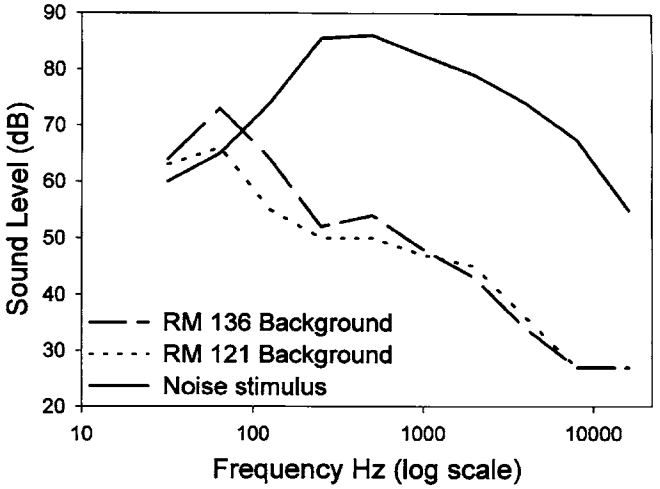


**Figure 1.** Frequency characterization of background noise and noise stimulus (ambient and experimental octave bands).

Ambient and experimental noise sound-pressure levels were measured with a calibrated microphone (type 4133, Bruel and Kjaer, Norcross GA), sound-level calibrator (type 4230, Bruel and Kjaer, Norcross, GA) and variable filter (model 3202, Krohn-Hite, Brockton, MA). This equipment enables the measurement of sound-pressure levels for frequencies <20 Hz to >20 kHz. The microphone was mounted on a tripod to avoid effects (acoustical shadows) of people within the room and was rotated in both horizontal and vertical planes to give an average reading. The microphone orientation had little effect on the readings, suggesting a homogeneous sound field within the rooms. Figure 1 demonstrates that the background noise had a similar amplitude (about 50 dB) and frequency distribution in both rooms. In this way, the rats were exposed to a noise stimulus or background noise.

A 3rd group of rats were housed in the noise room for 3 wk and then moved to the quiet room for a further 3 wk, to determine whether noise-induced effects on the intestinal mucosa could be reversed. These rats are referred to as the 'recovery' rats. In addition, the initial experiment was repeated but with the noise generator moved to the other room so that any possible effects of differences between the 2 rooms could be accounted for. Surgeries always were performed at the same time of day (starting at 08:00), to avoid chronobiologic variation.

**Experimental protocol.** Experiments were performed to determine the effect of noise on behavior (frequency of grooming, rearing and yawning), degranulation of mucosal mast cells, migration of eosinophils from the blood into the lamina propria, secretion of goblet cells, mean width of villus lamina propria, integrity of the mucosal epithelium, and fenestration of mucosal capillary endothelium (8 rats per group). Mucosal mast

cell degranulation, presence of eosinophils, secretion of goblet cells and villus width were evaluated by light microscopy after staining with toluidine blue. Epithelial integrity and endothelial fenestration were determined by electron microscopy,

**Observation of behavior.** We observed the behavior of 6 rats in the noise room and 6 in the quiet room (2 per cage) at night (between 20:30 and 22:00) on 4 occasions during the 3-wk period. Each rat was videotaped with an infrared camera for 2 min on each occasion, and the tapes were analyzed later to determine the time period during which and the number of times each rat performed 1 of 8 different behaviors: 1) sleeping on cage floor; 2) sitting still on cage floor while awake; 3) moving on cage floor; 4) grooming self; 5) grooming cagemate; 6) rearing; 7) interacting with cagemate (other than grooming); and 8) yawning. Behaviors 1 and 2 were classed as inactive, and behaviors 3 to 8 as active. Excessive time spent on behaviors 4 to 8 is stress-related.[22,37] Behavior 7 often included fighting.

**Intestinal surgery and preparation for microscopy.** The following protocol was reviewed and approved by the University of Arizona Institutional Animal Care and Use Committee. To prepare the small intestine for microscopy, the portal vein was incised for use as an outlet, the intestinal microvasculature was perfused at physiologic pressure with physiologically buffered saline, and the anesthetized animal was euthanized. The saline then was replaced with fixative. This procedure was performed so that the mucosal capillaries were flushed clear of red blood cells, to facilitate measurement of endothelial fenestrae.

Rats were anesthetized using intramuscular ketamine (20 mg/100 kg body weight) combined with acepromazine (1.25 mg/100 kg body weight), followed by intraperitoneal injection of sodium pentobarbital (6 mg/100 g body weight). When the rat reached a suitable level of anesthesia (no eye or foot reflex), a tracheotomy was performed so that the animal could be artificially ventilated. A midline incision from the pubis to the sternum was performed. Then, a gauze platform was built and saturated with N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid-buffer (HEPES) saline (pH 7.4) at 37 °C. The intestine was carefully externalized, wrapped in HEPES-soaked gauze, placed on the platform, and kept warm and moist with a constant drip of HEPES at 37 °C. The abdominal aorta was exposed, and a cannula was placed retrograde, distal to the superior mesenteric artery. The aorta was clamped just proximal to the superior mesenteric artery, and the circulation was flushed free of blood with approximately 5 ml HEPES. The animal was killed by intravenously injecting 0.5 ml Beuthanasia (Schering-Plough, Omaha, NE), and the HEPES was replaced by phosphate-buffered Karnovsky fixative (pH 7.4) for perfusion fixation. Pressure was maintained at 40 mm Hg, and the portal vein was clamped. Fixative also was applied to the outside of the intestine.

After 1 h, a segment of distal ileum (about 8 cm long) was excised and divided into 4 portions, each containing a Peyer patch. One portion was used for light microscopy and 3 for electron microscopy (randomly assigned). Each portion was further divided into the region with and without the Peyer patch; 4 portions with and 4 without Peyer patches were tested from each animal to ensure reproducibility. All portions were placed in fixative for an additional hour. The tissue segments then were rinsed in 0.2 M sodium cacodylate buffer and incubated overnight in 2% diaminobenzidine (DAB) at room temperature in the dark. Diaminobenzidine stains specific granules in eosinophils and thus helps in cell identification.

The following day, the segments were immersed for 1 h in DAB solution containing $H_2O_2$ to a concentration of 0.2%, rinsed 3 times in distilled water, postfixed in osmium tetroxide, and dehydrated and embedded in Spurrs resin for electron microscopy. The segments were oriented in the embedding medium so that the capillaries would be sectioned perpendicular to their length. This orientation was possible because in the intestinal mucosa, the capillaries are oriented subjacent to the epithelium and usually run parallel to the length of the villi. After sectioning, the grids were stained with uranyl acetate and lead citrate, which are general-purpose stains for membranous structures and nucleic acids.

**Light microscopy.** For light microscopy, thick (2 µm) sections cut longitudinally through the intestinal villi were stained with 1% toluidine blue (pH 7.0) for 15 min to identify mast cells under low-power (×10 objective) microscopy using an Axioplan microscope (Zeiss, Germany) equipped with 20× (numerical aperture, 0.6) and 40× (numerical aperture, 0.75; water immersion) Zeiss objectives. Toluidine blue is a stain that demonstrates the strongly sulfated mucopolysaccharide content of mast cell granules; mast cells stain red-purple, and the background stains blue. The total numbers of degranulated mast cells, eosinophils, and secreting goblet cells in each villus cross-section were counted in 10 to 15 villi per animal and then averaged. Degranulated mast cells were easy to identify by light microscopy because their remaining granules stained intensely with toluidine blue, and they also demonstrated empty vacuoles. Eosinophils had blue nuclei and dark-brown granules. Edema was quantified by measuring the width of villus interstitium between the epithelium on each side of the villus section. Each measurement was taken midway down the length of each villus. Only villus sections that contained a central lacteal were included, because they were centrally sectioned. For villus measurements, 4 sections were examined from each experiment from 2 different regions of the tissue sample. Each section usually contained about 10 villi. Measurements were made using tissue sections from 4 rats for each of the 3 groups. The measurements were averaged for each animal and these individual averages were combined into group means. For all histologic measurements, the investigator was blinded regarding the group from which tissue was derived.

**Electron microscopy.** We obtained 20 electron micrographs of sections from each segment by using an electron microscope (model CM12, Phillips, FEI Company, Tacoma, WA) at low magnification (×3000) to record the condition of the mucosal epithelium. The condition of the epithelium was assessed by noting whether most cells were intact or separated from each other or separated from the basement membrane. The presence (or absence) of eosinophils (identifiable by their bilobed nuclei) and mast cells in the micrographs was noted.

We examined the endothelial fenestrae in 10 capillary cross-sections per rat at a magnification of ×17,000. The fenestrae fell into 3 groups (Figure 2): 1) single-diaphragm fenestrae, in which a single, very distinct diaphragm covered the fenestra and the adjacent endothelium was thin; 2) double-diaphragm fenestrae were slightly shorter than single-diaphragm fenestrae, the 2 diaphragms present were not as distinct as those seen in single-diaphragm fenestrae, and the adjacent endothelium was 15% thicker; 3) transition-diaphragm fenestrae lacked distinct diaphragms, and a fuzzy layer was present where each diaphragm would be normally; the adjacent endothelium was 50% to 75% thicker than for single-diaphragm fenestrae. We counted the number of each fenestra type for each capillary cross-section and calculated the average counts for each animal.

**Statistical analysis.** Each parameter (numbers of degranulated mast cells, eosinophils, and secreting goblet cells; villus width; numbers of endothelial fenestrae; and behavioral measures)

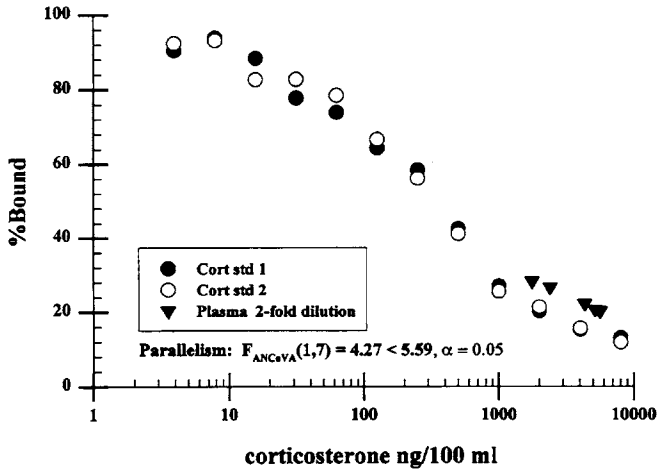


**Figure 2.** Electron micrograph of part of a transverse section through a capillary in the intestinal mucosa of a quiet rat. The endothelium shows the 3 types of fenestral diaphragms: single diaphragm (SF), double diaphragm (DF), and transition (or amorphous) diaphragm, (TF).

was compared between groups. Because most of the data for the various parameters (except behavioral) did not pass tests for normality or homogeneity of variance even when square root transformation was applied, we used nonparametric statistical tests. The Kruskal–Wallis test was applied for comparing different animals within the same group, and the Mann–Whitney Rank Sum test for comparing pairs of groups (for example, degranulated mast cells in noise versus quiet rats). Recovery rats were compared with those that remained in the noise room, and $n$ was the number of rats in a group. We determined the minimum number of animals per group to ensure statistical validity.[38] A stronger statistical test, one-way analysis of variance with blocking, was used on the mast cell data from the original study together with those from the replicate (rooms reversed) experiment to determine whether there was a greater difference between noise and quiet in one room versus the other (regardless of which room contained the noise generator).

## Results

**Effect of noise stress on feeding.** The daily period of white noise did not appear to affect the rats' feeding habits. At 4 wks after arrival, the weight (mean ± standard error of the mean) of a group of 8 noise rats was 404 ± 4 g after they had received 3 wk of daily noise (4 wk after arrival), and a group of 8 quiet rats that arrived on the same day as the noise rats weighed 419 ± 7 g 4 wk after their arrival. These 2 mean weights were not significantly different from each other.

**Effects of noise stress on behavior.** Noise and quiet rats showed a similar degree of overall activity at night, as evaluated by the percentage of time they spent in behavior types 4 to 8 (87% for noise rats, 80% for quiet rats). However, noise rats demonstrated significantly more grooming ($P < 0.01$) and rearing ($P < 0.001$) than did quiet rats (Table 1). As mentioned previously, these behaviors are indicators of stress.[22,37] In contrast, the frequency of yawning was not significantly increased in noise rats. When behavioral data means were calculated for each cage of rats undergoing a given treatment (that is, noise or quiet), there was less variation between cages ($P = 0.12$ for grooming, $P = 0.63$ for rearing) than between groups, indicating that the noise versus quiet treatment data were not confounded by different cages.

**Effects of noise stress on the structure of the overall appearance of the intestinal mucosa.** Upon visual inspection, the small intestine was more swollen and inflamed (reddish) in noise rats than in quiet rats. In addition, the Peyer patches were swollen along the whole length of the jejunum and ileum, indicating increased activation of the immune system.

**Table 1.** Time (mean ± standard error of the mean) spent by rats in active behaviors at night

| Group | Behavior | No. of episodes/2 min | % of total time |
|---|---|---|---|
| Noise rats | | | |
| | Grooming | 2.1 ± 0.5 | 7 ± 2 |
| | Rearing | 12.5 ± 1.0 | 60 ± 4 |
| | Yawning | 0.16 ± 0.08 | |
| Quiet rats | | | |
| | Grooming | 0.6 ± 0.2 | 6 ± 4 |
| | Rearing | 7.1 ± 1.0 | 47 ± 6 |
| | Yawning | 0.13 ± 0.07 | Not determined |

Behaviors were scored for 6 animals per group, 4 observations per animal. Data for the 4 observations for each behavior were averaged for each animal, giving 6 data points for each behavior. Data are reported as mean ± the standard error of the mean for the 6 data points per group.

**Light microscopy.** Longitudinally cut thick sections of parts of villi from a quiet rat and a noise rat are shown in Figures 3A and 3B, respectively. An intact mast cell, identified by its stained granules, in the lamina propria and adjacent to the central lacteal (CL) can be seen in Figure 3A. Degranulated mast cells (DMC) in the lamina propria can be seen in Figure 3B. There were significantly ($P < 0.001$) more degranulated mast cells per villus cross-section in the 10 villi closest to each edge of each Peyer patch in noise rats (3.95 ± 0.80, 60 villi) than in quiet rats (0.35 ± 0.29, 80 villi). The Kruskal–Wallis test demonstrated that there was much greater variance between groups than within groups ($P = 0.06$). Therefore the results were not confounded by differences between the cages. Compared with noise rats, recovery rats did not show a significant reduction in the number of DMC (2.37 ± 0.83, 115 villi). Corresponding numbers of DMC in villi within intestinal segments away from Peyer patches were 0.78 ± 0.33 (91 villi) for noise rats, 0.27 ± 0.19 (74 villi) for quiet rats, and 1.98 ± 0.79 (121 villi) for recovery rats. In this case, there was no significant difference between any 2 pairs of groups. Noise rats showed significantly fewer DMC in regions away from Peyer patches compared with those adjacent to those regions ($P < 0.01$; similar results were obtained when the noise and quiet rooms were reversed). Villi near Peyer patches showed 2.77 ± 0.72 DMC for noise rats and 0.39 ± 0.48 for quiet rats; corresponding values for villi away from Peyer patches were 0.45 ± 0.48 and 0.29 ± 0.40, respectively. One-way ANOVA with blocking demonstrated a significant ($P < 0.02$) difference between noise and quiet groups but not between rooms ($P = 0.14$), indicating that the data were not confounded by intrinsic differences between the rooms themselves.

In the lamina propria, villi near Peyer patches had significantly more eosinophils per villus section in noise rats (9.46 ± 0.44, 60 villi) than quiet rats (4.58 ± 0.38, 60 villi). Unlike mast cell degranulation and eosinophil migration, noise did not have any consistent effect on goblet cell secretion. Mean numbers of secreting goblet cells per villus cross-section adjacent to Peyer patches were 1.18 ± 0.59 for noise rats, 3.60 ± 2.35 for quiet rats, and 8.26 ± 2.00 for recovery; corresponding averages away from Peyer patches were 0.76 ± 0.58, 0.53 ± 0.47, and 2.44 ± 1.07, respectively. Comparison of the data revealed only one significant difference—adjacent to Peyer patches, there were more secreting goblet cells in recovery rats than in noise rats.

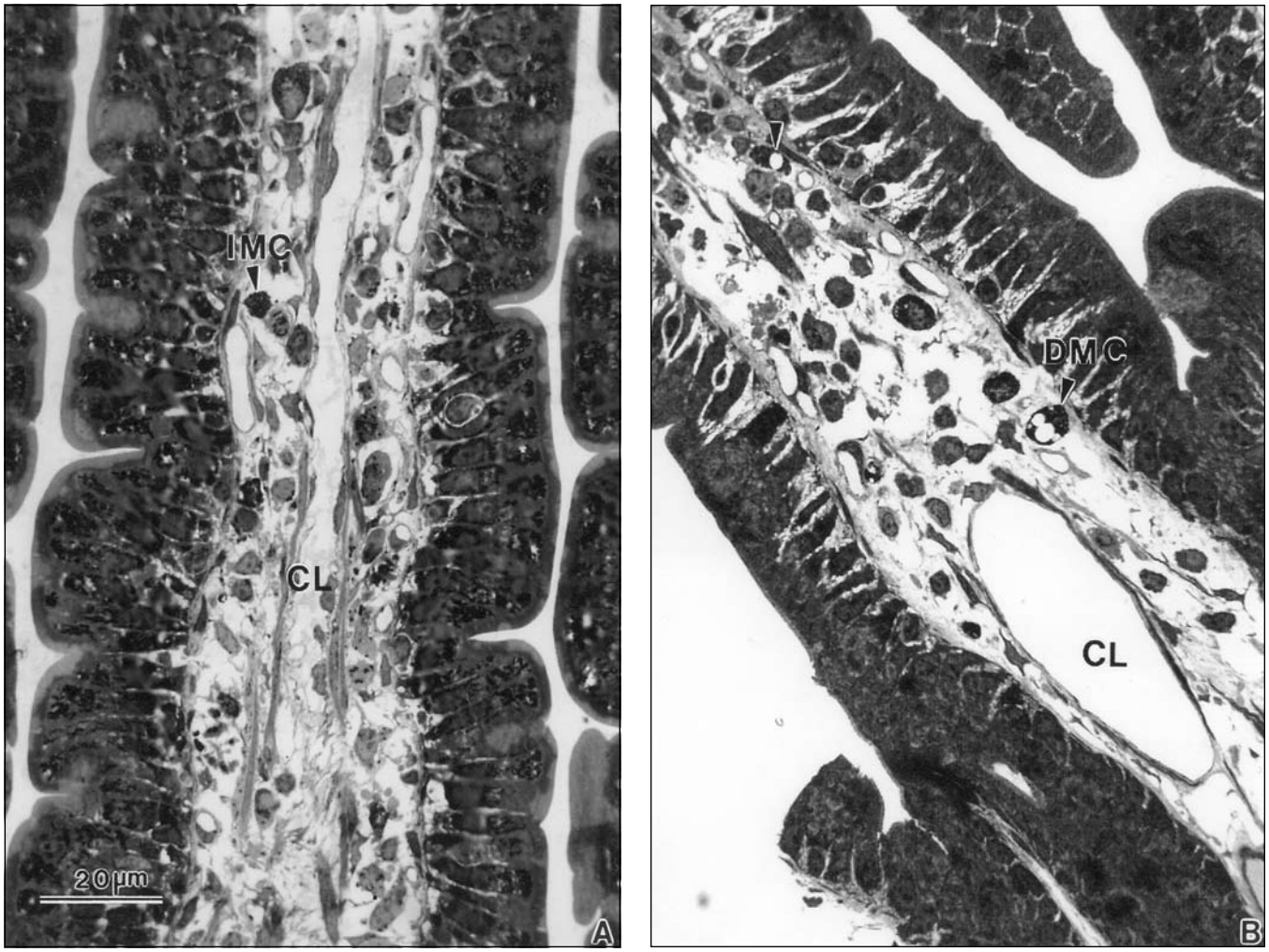


**Figure 3.** Light micrographs of longitudinally cut thick sections of parts of villi from a quiet rat (A) and a noise rat (B). The sections were stained with toluidine blue. (A) An intact mast cell (IMC), identified by its stained granules, in the lamina propria and adjacent to the central lacteal (CL) can be seen. (B) Degranulated mast cells (DMC) in the lamina propria are present.

Overall, the intestinal villi from noise rats were significantly more edematous than those from quiet rats, as assessed by measurements of villus lamina propria width using light microscopy. Mean villus width (± SEM) for each of the 3 groups (noise, quiet and recovery) were 57.0 ± 0.9 µm, 39.0 ± 0.7 and 59.0 ± 0.7, respectively. The distended central lymphatic vessels in villi from noise rats (compare CL between Figures 3A and 3B) and the greater area of cell-free tissue indicate that the increased width of the villus lamina propria was due to edema rather than to increased cell growth. The villi of the recovery group were just as edematous as those from the noise group, consistent with the finding that the number of DMC also remained high in this group.

**Electron microscopy.** Representative photomicrographs of the mucosal epithelium from the 3 groups of rats are shown in Figures 4A through 4C. Figure 4A demonstrates that in quiet rats, epithelial cells generally were attached to each other and to the basement membrane. Very few eosinophils were evident. Noise rats (Figure 4B), in contrast, usually demonstrated large numbers of epithelial cells that were separating from each other and, in places, were separated from the basement. In addition many intestinal villi contained eosinophils and partially degranulated mast cells. Figure 4B also shows an interepithelial leukocyte and capillary. Three weeks in the quiet room, following 3 wk in the noise room, produced some epithelial repair (Figure 4C). Although the epithelial cells were still somewhat separated from each other and had long, tenuous cytoplasmic projections from their junctional aspects, they rarely were separated from the basement membrane.

As described previously in the Methods section, endothelial fenestrae of the mucosal capillaries showed 3 different morphologies (Figure 2), which were all 3 groups of rats. A fenestra with a single diaphragm, another with a double diaphragm, and a 3rd with a transition diaphragm were visible in the same endothelial cell. The total average number of fenestrae per transverse cross-section of capillary for each of the 3 groups of rats was 16.47 ± 2.32 (60 sections) for noise rats, 14.57 ± 2.66 (100 sections) for quiet rats, and 18.90 ± 2.97 (70 sections) for recovery rats; these numbers were not significantly different from each other. The corresponding numbers for single-diaphragm fenestrae were 0.50 ± 0.45 for noise rats, 0.71 ± 0.61 for quiet rats, and 0.67 ± 0.55 for recovery rats. The average number of double-diaphragm fenestrae was 0 for noise rats, 0.04 ± 0.08 for quiet rats, and 0.03 ± 0.09 for recovery rats; neither the single- nor double-diaphragm fenestrae showed significant differences in number between the 3 groups. Although the recovery group appeared to show more transition fenestrae than did the quiet group, this difference was not statistically significant. The average number of transition fenestrae was 15.97 ± 2.25 for noise rats, 13.82 ± 2.63 for quiet rats, and 18.20 ± 2.80 for recovery rats.

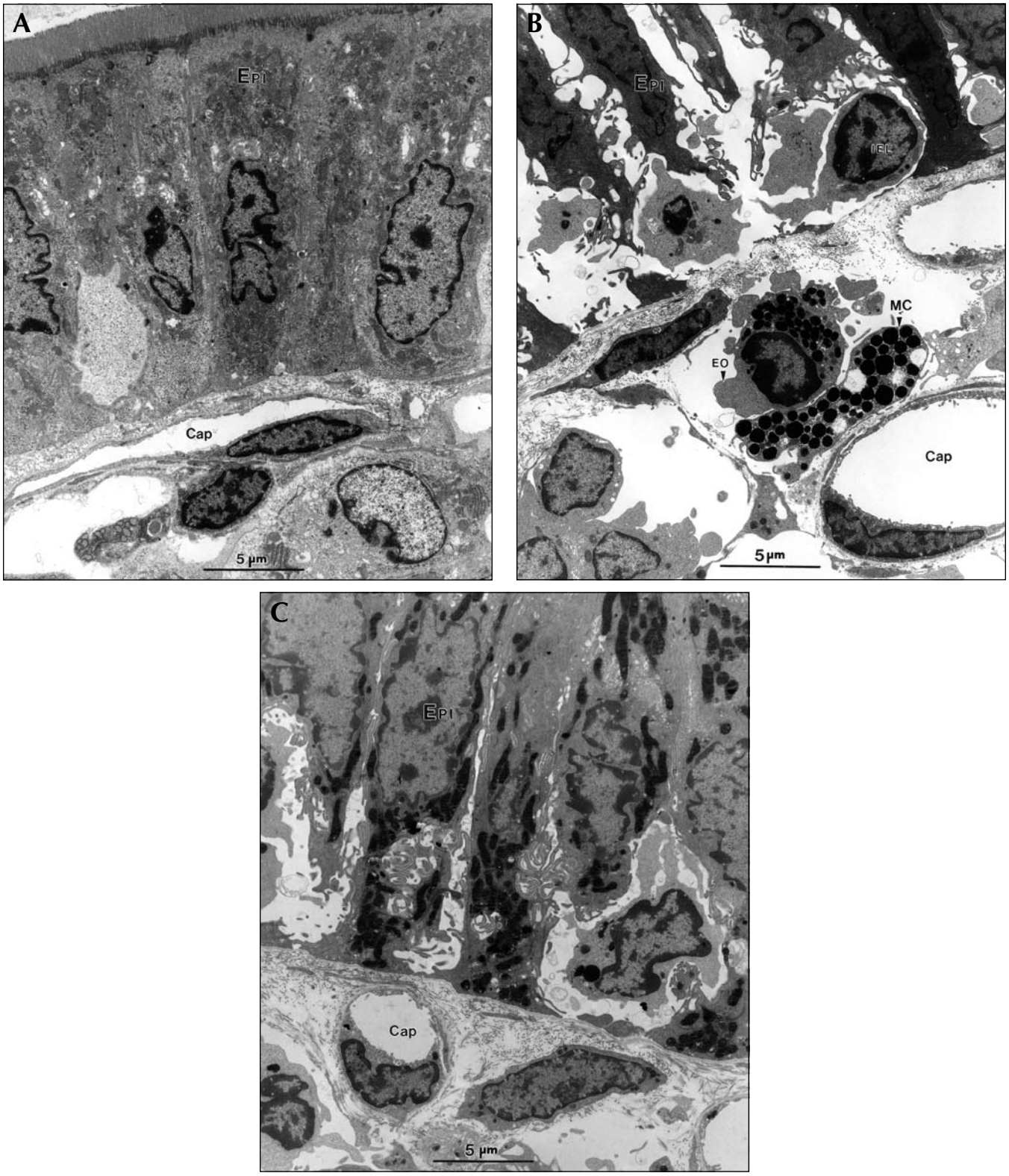


**Figure 4.** Representative electron micrographs of the mucosal epithelium from the 3 groups of rats. (A) In quiet rats, the epithelial cells (Epi) generally were attached to each other and to the basement membrane. Very few eosinophils were evident. (B) Noise rats usually demonstrated large numbers of epithelial cells that were separating from each other and that were, in places, were separated from the basement. Many intestinal villi contained eosinophils (EO) and partially degranulated mast cells (MC). An interepithelial leukocyte (IEL) and capillary (Cap) also are visible. (C) Returning rats to the quiet room for 3 wk after they had spent 3 wk in the noise room resulted in some epithelial repair.

## Discussion

This study demonstrates for the first time that daily exposure to a short duration of noise at an intensity that is encountered in many research animal facilities in the United States causes degranulation of mucosal mast cells, disruption of the epithelium, and edema of intestinal villi in laboratory rats. It is not clear whether this response is mediated via the hypothalamic–pituitary–adrenal axis, because accurate measures of

plasma corticosterone concentrations before and during the noise could not be obtained without causing further stress to the animals. Riley[29] obtained plasma corticosterone values of 60 to 300 ng/ml from unanesthetized rats that were agitated by the capturing procedures and then bled by the orbital technique. Guha and colleagues[14] reported plasma concentrations of 300 to 450 ng/ml in their control rats, which were not subjected to noise. These values are high and may have resulted from stress during handling; however, no information was given concerning how the blood samples were taken. In contrast, the basal corticosterone concentration of the rats exposed to noise in the study by Windle and coworkers[37] was 46 ng/ml. It is interesting to note that in that study, blood was automatically sampled by an indwelling catheter for measurement of corticosterone, and thus no handling was involved in procuring the samples. Windle and coworkers found that plasma corticosterone concentrations in rats varied periodically throughout the day; these levels significantly increased in response to 114 dB noise for 10 min if the onset of the noise coincided with the rising phase of a basal corticosterone pulse. This result suggests that the intestinal responses we observed in the present study in response to noise may have been a stress response that was mediated via the hypothalamic–pituitary–adrenal axis. The incidence of increased stress-correlated behavior that we observed in the noise rats compared with the quiet rats is consistent with this idea, but it is still possible that these differences in behavior are unrelated to stress.

As mentioned previously, increased permeability of the epithelium of the jejunum has been reported in rats subjected to noise.[6] Bijlsma and colleagues[6] attributed the increased permeability to endocytosis because they did not observe leakage of tracer material through the cell junctions. This result is inconsistent with our observation of noise-induced widening of epithelial cell junctions. However, Bijlsma and colleagues used a different noise protocol from that we used in the present study, and, most importantly, they did not examine tissue in the vicinity of Peyer patches. Gastrointestinal pathology also occurred in rats subjected to deliberate restraint,[16] activity stress,[1] or attack stress.[19] These findings demonstrate that the intestine is sensitive to stress responses produced by different stressors. The fact that the short bursts of white noise used in the present study produced similar effects to those resulting from restraint, attack, or activity implies that they also were sufficient to induce a stress response.

Before we consider the implications of our findings with regard to research that is performed using laboratory animals, we compare our current results with those reported in our previous study, in which rats were subjected to high or low personnel activity in the animal facility.[36] In that study, rats subjected to high personnel activity showed significantly greater mucosal mast cell degranulation and epithelial disruption than did those subjected to low personnel activity. These responses are similar to those produced by the daily exposure to white noise in the present study and suggest that a leading reason why high personnel activity caused deleterious effects was the excess noise involved. The 2 studies also are similar in that quiet (or low personnel activity) housing after noisy (or high personnel activity) housing did not reduce the number of degranulated mast cells counted. This finding suggests that a time period of 3 wk is insufficient for rat mast cells to regenerate and is consistent with a previous study in which rat peritoneal mast cells were still degranulated 56 d after stimulation.[20]

Two responses to high personnel activity that were not reproduced by subjecting the rats to white noise were goblet cell secretion and alterations in fenestral morphology. With regard to goblet cell secretion, the values for villi adjacent to Peyer patches in the quiet and recovery rats in the present study were very similar to their counterparts in the high–low personnel study. However, chronic exposure to white noise, unlike high personnel activity, did not increase goblet cell secretion. With regard to fenestral morphology, the slight increase in total numbers of fenestrae, which mainly was accounted for by an increase in multilamellar fenestrae, produced by high personnel activity was not observed in rats subjected to white noise. Therefore, mast cell degranulation, eosinophil recruitment into villus lamina propria, and epithelial disruption appear to be robust markers for the stress response in rats, whereas goblet cell secretion and changes in endothelial fenestral morphology are more complex reactions that may involve combination of different stimuli.

In our previous study[36] we hypothesized that stress-induced changes in the intestine, such as epithelial disruption, might be caused by excess reactive oxygen species (ROS). Activated phagocytes, such as neutrophils, eosinophils, and macrophages, are the best recognized sources of free radicals. In the present study, light and electron micrographs revealed significantly increased numbers of eosinophils in the lamina propria of villi from noise rats compared with those from quiet rats. Increased numbers of eosinophils probably were recruited from the blood into the intestinal mucosa of noise rats because of the additional increased presence of degranulated mast cells. Activated mast cells can release interleukin 5 which attracts eosinophils, particularly to Peyer patches.[21] Other products produced by mast cell degranulation then may stimulate eosinophils to produce leukotrienes and ROS.[28,32,39] In fact our electron micrographs often demonstrated eosinophils and degranulated mast cells in close juxtaposition (Figure 4B). The ROS and other products released by eosinophils may be partly responsible for the epithelial disruption observed near the Peyer patches of noise rats.

The mechanisms inducing mast cell degranulation are not clear from the experiments performed in this investigation. It is possible that locally produced neuropeptides, such as substance P, act on and perturb mast cells, phagocytes, lymphocytes, and the contiguous vasculature. In fact nerve terminals are in direct contact with the plasma membrane of mucosal mast cells in the rat ileum.[24] Substance P is one of the primary neurotransmitters of nociceptive nerves that reside in the gastrointestinal mucosa, and it has been shown that stress promotes release of substance P from the peritoneal tissues into the peritoneal cavity.[9] In addition, mast cell degranulation may be exacerbated further by ROS produced from eosinophils that the mast cells, themselves, have activated or by lymphocytes in Peyer patches. In fact, the stimulation of lymphocytes in Peyer patches and their subsequent release of cytokines could explain why mast cell degranulation was more prevalent at Peyer patches.[5] Results from a previous study suggest that ROS can act as stimulators of mast cells, at least in burns.[31]

The results of our current study have 2 important implications. First, they emphasize the importance of recognizing the nonauditory effects of noise in housing facilities for research animals. In the case of rats, this recognition is complicated by the fact that rats are very sensitive to sound in the range of 20,000 to 30,000 Hz, which is inaudible to humans[26] and must be monitored using an ultrasound detector. In a study by Sales and colleagues,[30] 24 of 39 sources of sounds in animal facilities and laboratories emitted ultrasonic sounds. Sources of ultrasound included cage washers, hoses, running taps, squeaky chairs, rotating glass stoppers, and oscilloscopes. Obviously

these sources will vary temporarily, spatially, and between institutions. Because even audible noise produces an inflammatory response in the intestine of rats, these environmental factors will confound the interpretation of experimental data related to gastrointestinal structure and function. In the report of a symposium on environmental and genetic factors affecting laboratory animals,[17] it was stated that "a common error in basic science research is the assumption that the animals used in different laboratories or under varying environmental conditions are all similar." The present study illustrates the relevance of that remark more than 25 y later and demonstrates that by closely controlling even a single environmental factor, the state of the intestinal mucosa of control animals can be improved significantly.

A 2nd implication of this study pertains to the nonauditory effects of noise in other species. For example, in humans it has been shown that a 10-min period of intermittent acoustic stress significantly increases leukocyte and lymphocyte counts and elevates B and T suppressor cytotoxic lymphocytes.[15] Excessive noise in healthcare settings can induce headaches, cause irritability, prolong wound healing, and increase sensitivity to pain.[7] Therefore it is important that conditions in hospitals should be optimized for patients' well-being and recovery. Determination of the brain-to-gut pathway by which noise affects the intestine is essential to inhibit deleterious gastrointestinal responses to noise stress both in animals and humans.

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