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ORIGINAL ARTICLE

The Influence of Environmental Exposure to Formaldehyde in Nasal Mucosa of Medical Students during Cadaver Dissection

Minako Hisamitsu[1], Yoshitaka Okamoto[1], Hideaki Chazono[1], Syuji Yonekura[1], Daiju Sakurai[1], Shigetoshi Horiguchi[1], Toyoyuki Hanazawa[1], Nobuhisa Terada[2], Akiyoshi Konno[3], Yoshiharu Matsuno[4], Emiko Todaka[4] and Chisato Mori[4]

ABSTRACT

Background: Environmental exposure to formaldehyde is commonly associated with clinical symptoms such as mucosal irritation and olfactory disorders. However, the impact of such exposure on the development of mucosal inflammation and its outcome has not been carefully evaluated.

Methods: The observational non-comparative study was planned. The study population consisted of group of 41 medical students who had signed up for a cadaver dissection course as part of their gross anatomy teaching at the school of medicine Chiba University in Japan. During such dissection course, the students are exposed to variable levels of environmental formaldehyde routinely employed for the preservation the cadavers. The subjects were evaluated by a detailed medical examination. We measured their serum IgE levels. In addition, an olfaction test and nasal mucosal sensitivity to histamine was serially determined, immediately before and after the course and 6 months after the completion of the course.

Results: Olfactory abnormalities were observed in 13/41 (32%) subjects and increased nasal mucosal hypersensitivity to histamine was observed in 17/41 (41%) during and immediately after completion of the course. These subjects had evidence of preexisting allergic rhinitis. 6/41 (15%) other students with no prior evidence of allergic rhinitis also exhibited formaldehyde associated clinical symptoms during the dissecting course. However, the symptoms disappeared upon completion of the course in all subjects studied.

Conclusions: Temporary abnormalities in the olfaction test and increased nasal mucosal hypersensitivity to histamine were observed in a few students with preexisting allergic rhinitis after environmental exposure of high concentrations of formaldehyde. These effects appeared to be transient.

KEY WORDS

clinical symptoms, formaldehyde, IgE, nasal hypersensitivity, olfactory disorder

INTRODUCTION

Formaldehyde is routinely used as preservatives for some food products and cosmetics. It is contained in common household cleaning products, adhesive agents for the wooden floors of buildings. In addition variable amount of formaldehyde is generated by certain natural generation sources.[1] Formaldehyde exposure has been associated with allergic diseases, "sick building syndrome", carcinogenesis such as pharyngeal cancer, lung cancer.[2-5] However, the mechanisms underlying such possible associations have not been fully explored to date. It is also not known, if environmental exposure to formaldehyde is associated

[1]Department of Otolaryngology, Head and Neck Surgery, [4]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, [2]E.N.T. Alliance, Chiba and [3]Otolaryngology Unit, Southern Tohoku General Hospital, Fukushima, Japan.
Correspondence: Minako Hisamitsu, Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Chiba University, 1–8–1 Inohana, Chuo-ku, Chiba 260–8670, Japan.
Email: hisamitsu–375@umin.net
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with any significant damage to external mucosal surfaces of the respiratory or gastrointestinal tracts. Cadaver dissection is an essential part of medical school training, and cadavers are routinely preserved in formaldehyde. It has been reported that formaldehyde-specific IgE antibodies are increased after exposure to formaldehyde. Little information is available about the dose, duration and nature of exposure underlying such increases in IgE activity.[6-8] In an earlier study using human nasal mucosal vascular endothelium, we found that exposure to formaldehyde enhanced the expression of adhesion molecules such as VCAM-1 and ICAM-1 and actually promoted the activation or migration of eosinophils in *in-vitro* settings.[9] The present studies were initiated in a group of Japanese medical students during their laboratory exercises in Anatomy in formaldehyde preserved human cadavers. The temporal effects of such exposure on olfaction and nasal mucosal hypersensitivity to histamine and on serum IgE concentrations was studied over a period of about 12 months.

METHODS

STUDY POPULATION

The study population consisted of the following groups of students (total 41, male26, female15), ranging in age from 21 to 25 years. Group A consisted of 17 students (males 8) with evidence of perennial mite-allergic rhinitis but without cedar pollinosis. 6 of them were classified as mild and 11 were as moderate-severe allergic rhinitis.[10] Group B consisted of another 8 subjects (males 7) with perennial allergic rhinitis and cedar pollinosis. All were moderate-severe. Group C comprised of 5 (males 5) with moderate-severe seasonal Japanese cedar pollinosis only with no other allergic rhinitis. Group D consisted of 11 students (males 6) no historical or clinical evidence of any allergic conditions (Table 1).

The student population was tested at regular intervals for nasal histamine sensitivity, nasal olfaction, and serum IgE levels. The diagnosis of perennial mite allergic rhinitis and pollinosis were based on clinical history, and the presence and the levels of serum mite or pollen specific IgE (CAP radio-allergosorbent test. CAP-RAST: SRL, Tokyo, Japan). There was no student with orchard grass pollinosis.

THE MEASUREMENT OF FORMALDEHYDE CONCENTRATIONS IN AN ANATOMY LABORATORY

Formaldehyde concentrations in the cadaver dissection laboratory in the School of Medicine at Chiba University during the course period were measured with a passive aldehyde samplers (SUPELCO DSD-DNPH, SIGMA-ALDRICH JAPAN, Tokyo, Japan) attached to the external clothing of each student as previously reported.[11]

Table 1 Student's profile

Allergic rhinitis	*n* = 30
Type of allergic rhinitis	
Mite perennial without pollinosis	*n* = 17 (Group A)
Mite perennial with cedar pollinosis	*n* = 8 (Group B)
Cedar pollinosis only	*n* = 5 (Group C)
Non-allergy	*n* = 11 (Group D)

STUDY PROTOCOL

This study plan was approved by the Ethical Review Board of the university, and was conducted after written agreement was obtained from each participating student. The cadaver dissection course was done from the beginning of April to the end of June in 2004. All subjects were evaluated by a careful medical examination, serum IgE levels, olfaction tests and nasal mucosal sensitivity to histamine were serially determined, immediately before and after the course and 6 months after the completion of the course (Fig. 1).

During the evaluation process, information was obtained regarding the presence or absence of any symptoms relating to ocular, nasal, pharyngeal, ear, skin, lower-respiratory, neurological, and digestive systems It was then determined whether the expression of any symptoms was associated only with the stay in the cadaver laboratory and if the symptoms persisted or resolved after leaving the laboratory at the end of each session.

Intranasal Examination

Intranasal examination was carried out to determine the presence or absence of increased nasal secretions and secretions and of any mucosal swelling

IgE Levels

Measurement of total IgE levels in the serum were carried out by employing commercially available radio-immunosorbent test (RIST; SRL, Tokyo, Japan) method. Measurement of formaldehyde-, mite-, cedar-, orchard grass-, or Artemisia-specific IgE antibody levels was carried out by employing the commercially available reagent kits (CAP-RAST).

Olfaction Tests

Olfactory cognition threshold was measured via a Nagashima jet nebulizing olfaction test.[12-15] Using five types of bromine, the minimum concentrations at which one could smell the odor and was able to recognize the odor were measured as the detection threshold and cognition threshold, respectively.

Nasal Mucosal Hypersensitivity to Histamine

Histamine dihydrochloride (Nakarai Co., Ltd., Kyoto, Japan, pH 7.2) was dissolved in phosphate buffered

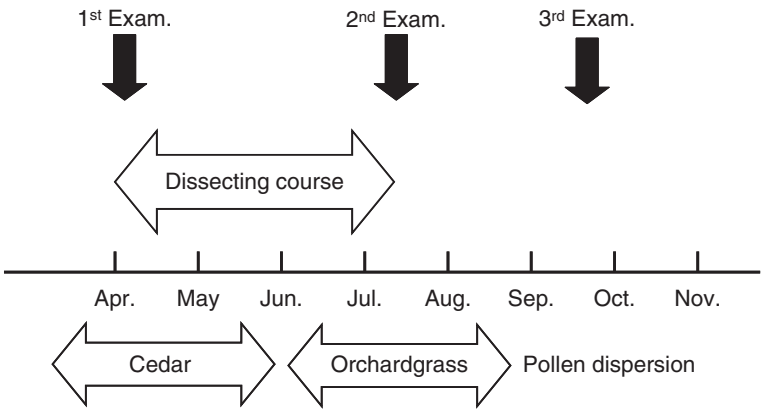

Fig. 1 Study protocol. The cadaver dissection course was done from the beginning of April to the end of June in 2004. All subjects were evaluated by a careful medical examination, serum IgE levels, olfaction tests and nasal mucosal sensitivity to histamine were serially determined, immediately before and after the course and 6 months after the completion of the course.

saline, and 20 μl aliquots of 10, 10^2, 10^3, 2.5×10^3, 5×10^3, 10^4 μg/ml concentrations were placed into the right and left lateral faces of the anterior edge of the mucosa of the inferior concha of the subjects. The subjects tilted their heads slightly downward and forward, and the number of sneezes within 10 minutes was measured. In addition to the amount of nasal discharge during the entire 10-minute period was measured by wiping the nasal discharge running from the anterior nares with tissue paper of a known weight and blowing their noses 10 minutes later.

As a fundamental study for the optimum concentration and the method of judgment, prior to this test, another 15 volunteer patients with moderate-severe perennial mite-allergic rhinitis (8 males, 7 females, 16-48 years of age) and 15 normal volunteers without any allergic rhinitis corresponding to the same age and gender were employed for the nasal histamine sensitivity examination. In addition, 5 volunteer patients with moderate-severe Japanese cedar pollinosis (20-35 years of age) were also included in the nasal sensitivity examination during the cedar pollen spread season (April) and off-season (June). These volunteers were different from the medical students in this study.

The students who had showed an increased olfactory cognition threshold or nasal mucosal hypersensitivity to histamine in June received these examinations again either 6 or 12 months later.

RESULTS

FORMALDEHYDE CONCENTRATIONS IN THE CADAVER DISSECTION LABORATORY

The dimensions of the anatomy laboratory were $15 \times 25 \times 3$ m and 1125 m^3, and there were 51 cadavers for the practical training. The formaldehyde concentration values measured by the passive method ranged from 0.51 to 0.97 ppm in the center of the laboratory, with an average concentration of about of 0.67 ppm. The concentrations in the corners, ranged from 0.22 to 0.70 ppm with an average of 0.44 ppm. These values are far higher than the guideline values set by the Ministry of Health, Labour and Welfare in Japan (0.08 ppm under general indoor conditions, 0.25 ppm in special work environments).

No significant differences in formaldehyde concentrations were observed at different times of the day or night or on different days of testing.

CLINICAL SYMPTOMS

Among the study population, 35 students (80%) complained of symptoms such as becoming sensitive to odors, smelling strange odors, eye irritation, ocular pain, burning in the throat, 15 students complained of poor body conditions such as headaches, heavy-headedness, 2 students complained of digestive symptoms such as abdominal pain, and 2 students complained of skin symptoms such as itchiness. Of these, 6 students exhibited the symptoms at least 50% of the time while in the cadaver dissection laboratory. However, the patients recovered after they left the laboratory. Two students were sensitive to odors, 2 students smelled strange odors, 4 students manifested eye irritation, and 2 students felt heavy-headedness. However, 6 months after completion of the dissecting course, these symptoms ceased in all 6 students. Similar symptoms did not occur in any of the subjects when entering any other buildings or at other locations in the campus under low concentrations of formaldehyde exposure.

Among these 6 students, symptoms of pre-existing allergic rhinitis existed in 3 students.

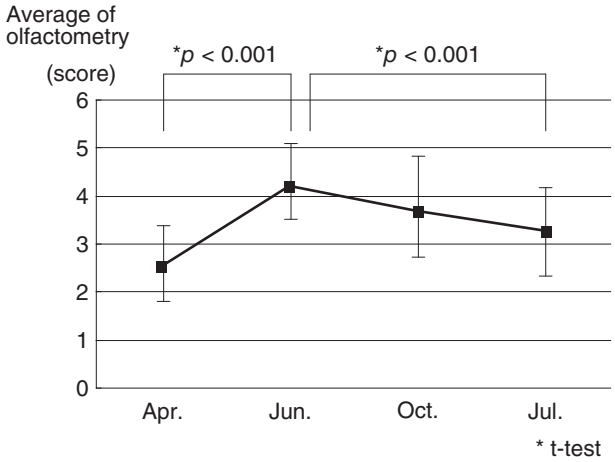

Fig. 2 The change of the cognition threshold before and after the anatomical dissection course. An increase in the cognition threshold was observed in 13/41 (32%) students in the second test. It means the aggravation of sense of smell. The impairments in smell improved 6 months later and mostly disappeared with complete recovery 12 months later.

On intranasal examinations, edema-like swollen mucosa or watery secretory fluid, which is characteristic of allergic rhinitis, was observed in some of the students, but no abnormal findings such as bleeding or scar formation were observed.

SERUM IgE LEVELS

The total serum IgE levels did not significantly change between the first to the third testing 12 months apart (data not shown).

The formaldehyde-specific IgE was detected only in one case. Other subjects did not exhibit any specific IgE response during or after completion of the dissection period. The FA specific IgE was detected only in one case at 1st examination. It did not increase and decrease, and lasted at 4th examination. No correlation existed between the severity of symptoms and formaldehyde specific IgE level (data not shown).

OLFACTION TEST

An increase in the cognition threshold was observed in 13 students in the second test. These all 13 students originally had preexisting history of allergic rhinitis. Of these, 2 subjects had cedar pollen allergies, 6 had perennial mite-allergic rhinitis, and 5 had evidence of cedar pollen and mite allergies. The impairments in smell improved 6 months later and mostly disappeared with complete recovery 12 months later (Fig. 2).

NASAL MUCOSAL HYPERSENSITIVITY TO HISTAMINE

The 15 volunteers with mite allergic rhinitis and 15 normal volunteers without any allergic rhinitis were subjected to intra nasal provocation with serially increasing concentrations of histamine (10, 10^2, 10^3, 2.5×10^3, 5×10^3, 10^4 μg/ml concentrations, diluted in phosphate-buffered saline). The number of sneezes and the amount of nasal discharge observed within the first 10 minutes and after 1 week are shown in Figure 3a, b, Figure 4 respectively. These experiments demonstrated that more than 2.5×10^3 μg/ml histamine solution significantly induced the amount of nasal discharge (Fig. 3a) and the number of sneezes (Fig. 3b). In addition, higher reproducibility was observed in the amount of nasal discharge than the number of sneezes (coefficient of variation ± 32.4% for sneezing and ± 21.6% for nasal secretion.) (Fig. 4). The results in the 5 cedar pollinosis patients suggested that the nasal sensitivity to histamine was as high as that of mite allergic patients during pollen season, however, it was as low as that of the normal volunteers in the off season periods (data not shown). As a result, the histamine reactivity in the medical students in this study with the amount of nasal discharge obtained by the administration of 20 μl × 2 (both nasal passages) of histamine at a concentration of 2.5×10^3 μg/ml as an indicator.

At the first test when the cedar pollen was in-season, the nasal sensitivity to histamine which was defined by the amount of nasal discharge was high in the Group B and Group C students who had cedar pollinosis than in Group D who were non atopic (Table 2). At the second examination in June when the cedar pollen season was over and just after the completion of the dissection course, the amounts of nasal discharge decreased in Group B compared with at the first examination but the decrease was not observed in Group C. Compared to the first test, the increased nasal sensitivity to histamine at the second test was observed in the Group A students with mite allergic rhinitis who were not allergic to either cedar pollen or orchardgrass (Table 2). In non-atopic students (Group D) a small amount of nasal discharge was obtained by the nasal administration of histamine and did not increase even when examined after the dissection course (Table 2).

The expression of the associated clinical symptoms during the dissection course described above was observed in 3 subjects who had increased nasal mucosal hypersensitivity to histamine. However other subjects did not exhibit any such hypersensitivity and no specific association with formaldehyde exposure could be identified. The increased nasal mucosal hypersensitivity observed in the allergic rhinitis students in June disappeared 6 months later (data not shown).

DISCUSSION

Environmental exposure to prolonged, but varying levels of formaldehyde in cadaver dissection settings resulted in the development of sensory symptoms

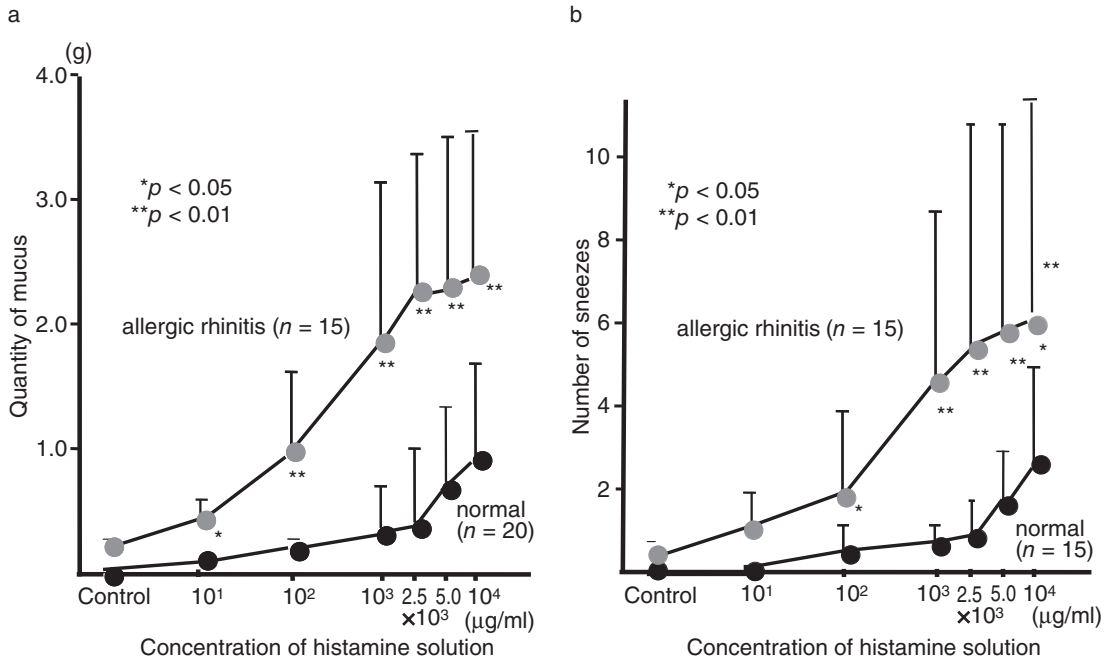

Fig. 3 **a**, **b**: The amounts of nasal discharge and the number of sneezes after a various concentrations of histamine in the perennial allergic rhinitis volunteer patients.

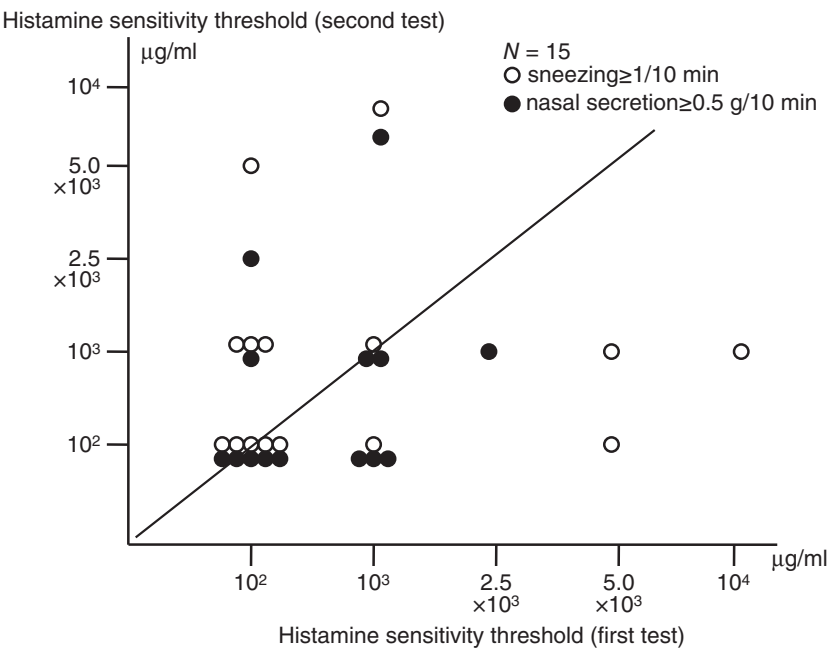

Fig. 4 The result of the histamine administration conducted for about one week in the volunteer patients with perennial allergic rhinitis. The coefficient of variation was ±32.4% in sneezing and ±21.6% in nasal secretion.

such as smelling of strange odors and eye irritation, and general symptoms such as nausea and headache in about 15% subjects. These symptoms were observed at a high frequency upon entering the laboratory, and expressed under conditions in which an association with formaldehyde exposure in the laboratory was evident. In general, these symptoms disappeared upon the completion of the study of dissection course and similar symptoms did not occur in other buildings or at other locations under low levels of for-

Table 2 The nasal mucosal sensitivity to histamine expressed by the amounts of nasal secretion

	1st exam.	2nd exam.	1st vs 2nd *p* value
Group A	0.678 ± 0.570 NS	1.090 ± 0.772	0.00628
Group B	1.865 ± 1.306 *	1.262 ± 0.922	0.01112
Group C	2.544 ± 1.207 **	1.766 ± 2.829	0.21756
Group D	0.760 ± 0.483	0.736 ± 0.638	0.4605

(t-test) * $p < 0.01$, ** $p < 0.05$.
NS, no significant.

maldehyde exposure.

Cadaver dissection related exposure did not result in any changes in the total IgE level or the production of formaldehyde-specific IgE. Wantke, *et al*. have demonstrated formaldehyde-specific IgE production in students after cadaver dissection courses.[8] In the present study, the effects of 3 months of exposure at high concentrations of formaldehyde was followed over a 12-month periods and little or no formaldehyde-specific IgE production was demonstrated. Although these limited studies in normal subjects, some with evidence of allergic rhinitis or other atopic disposition, do not rule out the possibility of the involvement of formaldehyde in IgE production, it appears that specific IgE mediated allergic reactions are uncommon after such limited environmental exposure.

As a method of assessing nasal mucosal hypersensitivity, measurement of the weight of nasal discharge obtained 10 minutes after the administration of 20 µl of 2.5×10^3 µg/ml histamine solution to the mucosa of the both concha nasalis inferior was considered as an easy way of differentiating and evaluating the nasal mucosal hypersensitivity of patients with allergic rhinitis, which is known to exhibit a typical disorder of nasal mucosal hypersensitivity, from of normal subjects without allergic rhinitis. Consequently, the nasal mucosal hypersensitivity of the students before and after the anatomy dissecting course was studied using the present method in order to clarify the effect of three months exposure to high concentrations of formalin. However, the effect of pollen allergens needed to be taken into consideration in the test of nasal mucosal hypersensitivity to histamine. The peak dispersal of cedar pollen, which is a typical pollen allergen in Japan, had ended by the beginning of May and the peak spread of orchard grass pollen could be seen from the mid May through the end of July. It could be assumed that hypersensitivity increased in the students with moderate/severe mite allergic rhinitis due to exposure to mite allergen all year around because of chronic allergen exposure. In the present study, one third of the students with mite allergic rhinitis without pollinosis had only mild symptoms of rhinitis and their nasal hypersensitivity was not significantly different from that of non-allergy subjects before the anatomy dissection course, however, the sensitivity was increased after the course. The sensitivity could be enhanced easily in the patients with pollinosis in pollen season and the increased sensitivity was observed in the students with cedar pollinosis in the first examination during pollen dispersal season. However, the increased sensitivity was not recovered in June under normal circumstances, two months after the end of cedar pollen season, which was just after the completion of the anatomy dissecting course, in comparison to April. The increased nasal sensitivity was not observed in the students without allergy even after the dissection course. These suggest that the exposure of high concentration of formaldehyde could influence on the nasal mucosa with allergic rhinitis and increase the sensitivity to histamine but not on the nasal mucosa from non-allergy.

The mechanism for this is unclear but the enhanced expression of molecules adhering to the nasal mucosal vascular endothelium due to formaldehyde and the increased adhering of eosinophils may exist, as reported previously in *in vitro* study.[9]

In the present studies, all subjects recovered from the increased hypersensitivity observed initially, and the effect on olfaction did not persist. Furthermore, no association was observed between the expression of clinical symptoms during the practical training, increased nasal mucosal hypersensitivity, and the abnormal olfaction. Based on this report, it appears formaldehyde exposure associated with cadaver dissection over a short period is not detrimental to the long term homeostasis of human mucosal surfaces.

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