PROTECTIVE ROLE OF HONEY AFTER LEAD TOXICITY EFFECT ON ALBINO RATS LINGUAL PAPILLAE

Waleed O. El shawakh[1], Afaf A. El-Sawa[2], Sahar K. Shafik[3]

ABSTRACT

INTRODUCTION: Studies concerning exposure of human to lead during daily activities are sometimes leading to many complications. Lead is a poisonous heavy metal, it's toxicity can change the antioxidant balance in biological tissues. Honey is rich in phenolic acids and flavonoids, which exhibit a wide range of biological effects and act as natural antioxidants.

OBJECTIVES: (1) To investigate the histological changes in lingual papillae following lead toxicity in experimental rats. (2) To assess the possible protective effects of honey using scanning electron microscopy (SEM).

MATERIALS AND METHODS: Thirty two adult male albino rats were divided into four groups, eight rats/ group. Group I (control): 1ml distilled water. Group II (control + honey): 50 mg/kg honey + 1ml distilled water. Group III (lead): 16.5 mg lead acetate. Group IV (honey + lead): 16.5 mg lead acetate + 50 mg/kg honey. All doses were given by oral intubations daily /6 weeks. After 6 weeks, rats were sacrificed, and tongues were dissected out and processed for SEM.

RESULTS: In Group II: Increased papillary density of the filiform papillae was noticed. In Group III the filiform papillae were distorted. Some appeared shorter while others had eroded tips and hyperkeratosis. Complete loss of papillae were seen in some samples. Disfigured fungiform papillae with swollen taste buds were seen. In Group IV, filiform and fungiform papillae appeared close to normal with minimal changes.

CONCLUSIONS: The atrophy observed in Group III could be explained by lead-induced oxidative stress. Lead toxicity had a dual effect where increased free radical formation together with depletion of endogenous antioxidant enzymes resulted in inflammation and increased cell injury. Lead causes anemia and decreased salivary secretion, which may explain the epithelial erosion. Honey exerted its protective role through restoring enzymatic activity and through its antioxidant mechanism.

KEYWORDS: Honey, filiform papillae, lead toxicity, fungiform papillae, SEM.

1- B.D.S. Faculty of Dentistry Triopli University
2- Professor of Oral Biology faculty of Dentistry Alexandria university
3- Professor of Oral Biology faculty of Dentistry Alexandria university

INTRODUCTION

The civilization advances and growing environmental pollution bring the effect of various xenobiotics, including heavy metals on the function of the living organism (1).

Lead is one of the most commonly used metals in industry and it has been shown that it serves many useful purposes. Lead toxicity is of concern to the public health as it has been documented as a cumulative environmental dangerous contaminants (2). So the extensive use of its compounds in various fields of industry and general life makes intoxication with this metal one of the most serious toxicological problems facing human health(3).

Many workers in the lead-based industries are not aware of the dangerous effects of lead and so do not take proper precautions while handling it, leading to higher levels of exposure.

Lead industries include; lead production and soldering, manufacturing of batteries, ceramics, plastics and bullets as well as metal radiator repair and recycling lead cables. Some construction work like demolition of old buildings, scraping of lead-based paint, repair of bridges, water tanks, welding of lead painted metal and furniture refinishing may also expose workers to considerable amounts of lead(4).

Oxidative stress is the main contributor to lead toxicity as lead directly and/or indirectly can change the antioxidant balance in biological tissues (5).

Although the biological mechanisms linking lead exposure and dental disease are not completely clear, it has been suggested that lead disrupts salivary gland function, thereby increasing the risk of dental caries. Studies on rats have shown that lead exposure during the prenatal and perinatal periods resulted in significantly higher levels of dental caries and markedly reduced salivary flow rate(6).Similarly, adult rats exposed to lead through drinking water showed decreased salivary calcium and protein concentrations, increased lipid peroxidation and a decrease in total antioxidant capacity in salivary gland tissue. This indicated the presence of lead-induced oxidative stress (7).

Regarding the oral manifestations of lead exposure in humans; ulcerative stomatitis, gray spots on the buccal mucosa, a heavy coating of the tongue surface, and tremor of the extended tongue have been documented (8). Bluish-red or deep blue linear pigmentation of the gingival margin may be present (9).In addition to mucous pigmentation, gingivitis, tongue burning, and reduced taste perception also occur (10). Clinical studies show that occupational lead exposure is a major cause of ulceration, fissuring and epithelial desquamation of the tongue, palate and other parts of the oral mucosa. Lead also increases the incidence of periodontal diseases (gingivitis and periodontitis), caries index and dental abrasions (11). The histological effects of lead exposure have been the focus of substantial experimental research throughout the years. However, the study of the histological effects of occupational lead toxicity on the lingual papillae has not drawn much attention.

It has been known since ancient times that honey has protective and curative properties (12). Honey is a remarkable liquid, prepared by honeybees from the natural solutions called nectar obtained from various flowers (13). Honey contains a number of enzymes (like catalase and glucose oxidase) and free amino acids. It also contains some vitamins like riboflavin (vitamin B2), ascorbic acid (vitamin C), minerals like iron and calcium, antioxidants (such as flavonoids, vitamin C, catalase enzyme, polyphenols) and antibacterial agents like hydrogen peroxide (14). Its high viscosity, acidic pH, high osmolarity, and rich nutritional properties can inhibit bacterial growth and enhance healing (15).

Honey is an extremely complex natural liquid that is reported to contain at least 181 substances. It is a supersaturated solution of sugars, of which fructose (38%) and glucose (31%) are the main contributors (16).

A study investigated the protective effect of honey against lead toxicity in rats. The results showed a significant decrease of lead uptake in blood and tissues and a marked recovery in the biochemical alterations caused by lead (16).

In humans, numerous filiform (hair like) papillae cover the anterior part of the tongue and consist of cone-shaped projections, each with a core of connective tissue and a keratinized epithelium. They are highly abrasive during mastication compressing the food bolus against the palate (17).

The rat filiform papillae have been classified into three distinct types by many researchers. However, another study defined the three types of filiform papillae on the rat tongue as simple conical papillae, giant conical papillae, and true filiform papillae (18).

The simple conical papillae cover the anterior two-thirds of the tongue (body of the tongue). They form a curved conical structure ending in a strong, cornified spine (19). The giant conical papillae form 7 to 10 rows, in the intermolar eminence and separate the simple conical papillae from the true filiform papillae. The true filiform papillae occupy the posterior one third of the tongue (base of tongue). All of the filiform papillae possess a convex as well as a concave surface (18).

The second type of the lingual papillae is the isolated fungiform (fungus like) papillae found scattered between filiform papillae. In human, they are elevated, mushroom-shaped papillae which appear red because of their relatively thin non-keratinized epithelium overlying a highly vascular connective tissue core. Taste buds are normally present in the epithelium of the superior surface (17).

The search was approved from the Research Ethics Committee of the Faculty of dentistry-Alexandria University.

MATERIAL AND METHODS

Animals

Thirty two adult male albino rats weighing between 180 g and 200g were kept under normal healthy conditions in the Animal House of the Medical Research Center in Alexandria University for the duration of the experiment. The rats were housed in wire mesh cages (four rats per cage) under controlled temperature and dark-light cycle. They were fed a standardized diet and tap water.

Materials

Lead: Inorganic lead acetate trihydrate powder (Sigma Chemical Company).and pure unprocessed honey (Imtanan health shop).

Experimental Design The albino rats were divided into four groups, 8 rats in each group. The groups were classified into:

- **Group I (control):** Animals were given 1ml distilled water daily by oral intubation.
- **Group II (control + honey):** Animals received 50 mg/kg of honey dissolved in 1 ml of distilled water daily by oral intubation.
- **Group III (lead):** Animals received 16.5 mg of lead acetate per day, which is equivalent to the occupational lead exposure (500 ppm) (20). The dose was given in 1ml of distilled water by oral intubation.
- **Group IV (lead + honey):** Animals received 16.5 mg of lead acetate each day followed directly by a honey dose equivalent to 50 mg/kg dissolved in 1 ml of distilled water by oral intubation (21).The duration of experimental study was six weeks. At the end of the 6 weeks the rats were sacrificed. Tongues were immediately dissected out and processed for scanning electron microscopic examination.

Scanning Electron Microscopic Preparation

Tongue specimens were fixed in buffered glutaraldehyde (2.5%glutaraldehyde in 0.1 Molar phosphate buffer) with a pH 7.2, for1.5-2 h, and then rinsed 2 times in phosphate buffer 15 min for each. The samples were placed in post-fixative (osmium in 0.1 Molar phosphate buffer) with a pH 7.2 for 2h. The traces of unbound osmium will be rinsed with two changes of buffer, 15 min for each.

Dehydration were then done in an increasing series of ethanol with concentrations (40-60-80-95-100%), 15 min for each step.The next step was drying by critical point drying. The final step in processing was the coating by Baltec 030 sputter coater (22). Specimens will be then examined by the SEM. The search was approved from the Research Ethics Committee of the Faculty of Dentistry-Alexandria University.

RESULTS

SEM examination of the filiform papillae showed:

The filiform papillae: Group I: These papillae appeared closely packed, thread-like in shape with a nearly regular orientation (Figure1A). Group II: Increased density of the filiform papillae was noticed (Figure1B). Group III: Decreased density of the filiform papillae was noticed here unlike the closely packed picture of Group I. Most papillae did not present the typical thread shape where most papillae appeared short and bent with hyperkeratotic tips (Figure2A). Group IV: Closely resembled that of Group I with a thread-like shape, regular orientation, normal interpapillary distances and no bending of tips (Figure2B).

SEM examination of the fungiform papillae showed:

Group I

The fungiform papillae appeared rounded with a smooth surface. A centrally located well-defined regular taste bud was seen surrounded by a shallow indentation

(Figure3). Group II: The fungiform papillae appeared more prominent and distinct. The surface was regular and the typical mushroom-shape was distinguished (Figure4). Group III: The fungiform papillae seemed less prominent and clearly distorted.

The surface seemed irregular and wrinkled and the typical mushroom-shape could not be distinguished. The taste buds on most of the fungiform papillae were rarely encountered as they appeared rather smooth and ill-defined

(Figure 5). Group IV: The fungiform papillae appeared regular with a well-defined outline, nearly similar to that of Group I. However, it was less projecting over the surface. The surface appeared smooth with a well-defined taste bud (Figure 6).

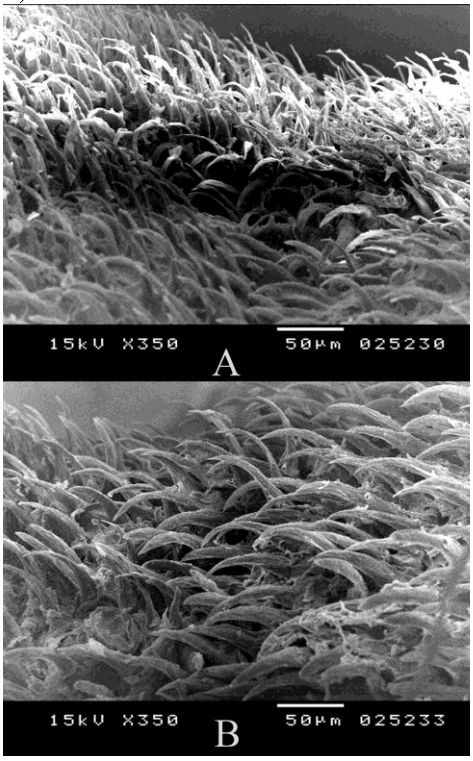

Figure 1: Scanning electron micrograph showing A) closely packed thread like true filiform papillae of Group I with a regular orientation (origina mag. x 350). B) longer and more curved and numerous of true filiform papillae of Group II (original mag. x350)

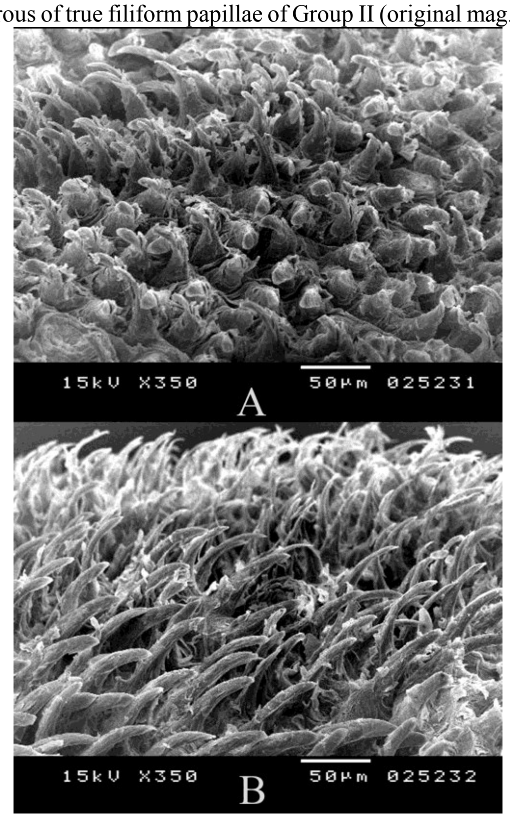

Figure 2: A) Scanning electron micrograph of the true filiform papillae of Group III showing decreased papillary density and loss of the typical thread shape. Most papillae appeared short, bent with hyperkeratotic tips (original mag. x350)
B) Scanning electron micrograph of thread like true filiform papillae of Group IV no bending of tip (original mag. x350)

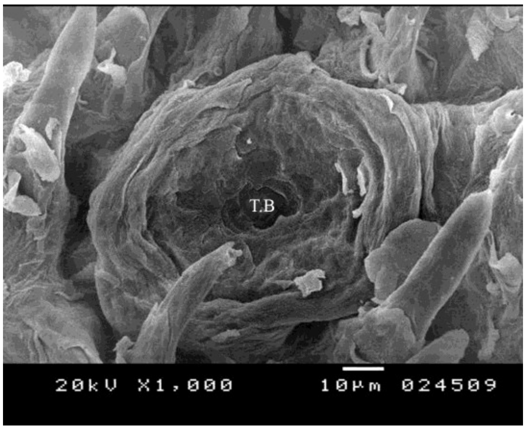

Figure 3: Scanning electron micrograph showing a fungiform papillae of Group I with a smooth surface aroud a centrally located well defined regular taste bud surrounded by a shallow indention (original mag. X1000).

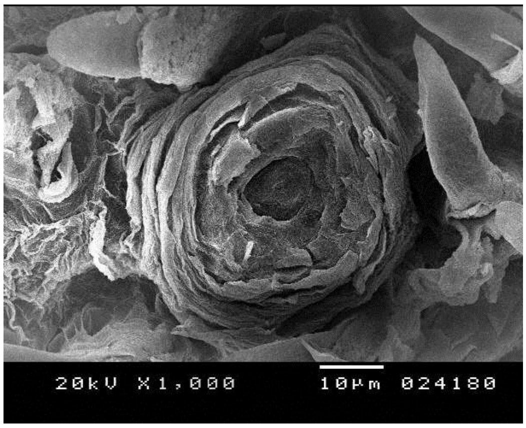

Figure 4: Scanning electron micrograph showing more aroud a centrally located well defined regular taste bud surrounded by a shallow indention of fungiform papillae of Group II (original mag. X1000).

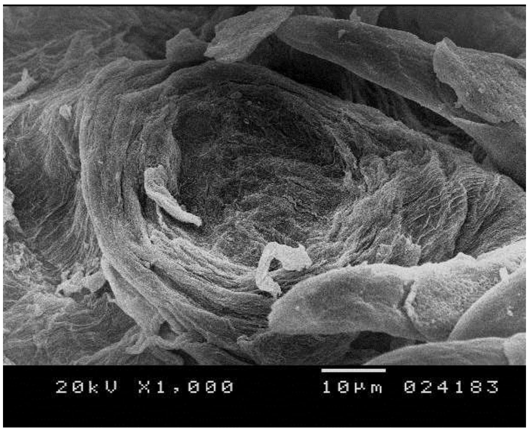

Figure 5: Scanning electron micrograph showing the clearly distorted fungiform papilla of Group III with a wrinkled surface. The taste bud appeared smooth and ill-defined (original mag. X 1000).

DISCUSSION

There has been a growing interest to evaluate the use of natural remedies to protect against day to day health problems. This was the inspiration behind the present study. Occupational lead exposure presents a serious health hazard to workers in lead- based industries. Great efforts are required to increase awareness of workers at risk. Adequate prevention programs demonstrating good occupational hygiene and emphasizing proper precautions taken during handling of this toxic metal are also needed (23).

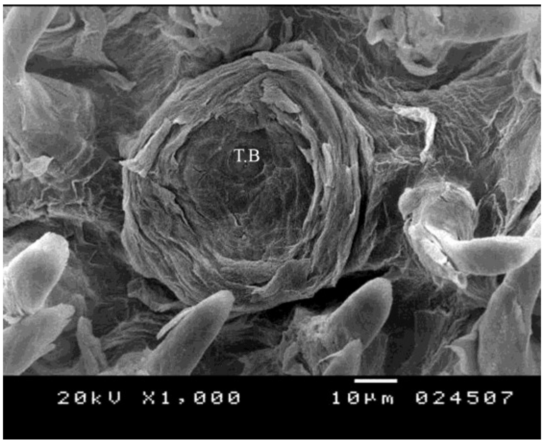

Figure 6: Scanning electron micrograph of fungiform filiform papillae of Group IV portraying a regular smooth surface, a well-defined taste bud (original mag. X1000).

In addition, the use of natural antioxidants has a great potential to protect against lead-induced toxic effects on tissues. If honey proves to be helpful in reducing these toxic effects, then many workers will benefit from regular consumption of such natural remedies without suffering needless side-effects of prescribed medication.

In the present study, the search for the appropriate lead was a challenge. In many previous studies, lead was added to the basal diets or drinking water of experimental rats. However this method was avoided since the exact amounts of leaded diet or water would vary among individual animals. It was thus preferred to standardize the lead dose given to each rat every day for the whole six-week duration of the experiment. The dose given to rats was 16.5 mg of lead acetate per day using oral intubation, which is equivalent to occupational lead exposure (500ppm) in workers as calculated by anderzejewska et al (20).

The tongue is considered as a mirror of the general health, especially the filiform papillae. Cells of these papillae have a high metabolic activity and so any enzymatic disturbance, vascular changes or nutritional deficiency result in their atrophy. They un dergo loss and atrophic changes faster and earlier before any other papillae (24).

Lingual atrophy may be a side-effect of a number of medications such as antibiotics, or may be due to cancer, diabetes, chemotherapeutic agents or metal toxicity (25). This is why lingual papillae were the tissue of choice in the current study.

The SEM results of the present work revealed atrophic changes in the filiform papillae of Group III.This atrophy appeared in the form of apparent thinning of papillae, tearing of keratin, decreased papillary density in addition to areas completely devoid of papillae. This is in agreement with a clinical study, which declared that chronic lead exposure caused ulceration and desquamation of the tongues of workers in lead-based industries (11).

Histological changes of filiform papillae included shortening, loss of thread shape and erosion of papillae tips, along with areas of complete epithelial erosion (26). Other studies also reported similar effects of lead on different tissues; where a dose of 100 ppm was given to rats in drinking water for 28 days. The results revealed desquamation, erosion and ulceration of the surface epithelium of intestinal villi. The investigators attributed this atrophy to the irritative effect of lead on the mucosa of the intestine (26).

This atrophy could be explained by lead-induced oxidative stress. Oxidative stress can be defined as an imbalance between the production of reactive oxygen species and antioxidant defense (27). Much of lead's toxicity can be attributed to distortion of enzymes and structural proteins (28). This can be further explained by a biochemical analyical study, which assessed the lead-induced oxidative stress on gastric ulcers (29). Lead was given to rats at a dose of 100mg/L in drinking water for 15 weeks. The results showed that lead exposure aggravated gastric ulcer by interfering with oxidative metabolism and increasing lipid peroxidation. It was found that lead has a dual effect as it increased free radical formation together with depletion of endogenous antioxidant enzymes which are free radical scavengers. Therefore, accumulation of free radicals resulted in inflammation and increased cell injury. This mechanism is well- known to cause cell injury in many tissues. (5, 30, 31) and may be the cause of the lingual atrophy observed in the current study.

It is worth mentioning here that patients with atrophic tongue are reported to suffer from lowered rates of saliva secretion (25).

A study investigated the oxidative effect of lead toxicity on rat submandibular gland and saliva using 100 mg/kg of lead acetate. After 2 h the gland and saliva were collected for evaluation. Results proved decreased secretary function and altered salivary composition, which were attributed to increased oxidative stress (32). Hence, it can be suggested that the indirect effect of lead on decreased salivary function may be another cause of the lingual atrophy seen in the present study.

Furthermore, another cause of lingual atrophy is anemia where changes like dryness of the mouth, glossitis and patchy atrophy of filiform papillae have been reported (33). A review on the effects of lead on the body revealed that lead causes impairment of detoxification of environmental toxins and reduces hemoglobin synthesis; thus, decreasing oxygen transport to all tissues, ultimately causing anemia (34). This is in agreement with another study, which stated that in chronic lead toxicity, anemia is a common clinical finding (35). Again this association may explain the atrophic changes observed in this experimental model where lead-induced anemia may have caused a similar picture to lingual atrophy in anemia.

It was reported that during the process of atrophy the filiform papillae are affected first, followed by fungiform papillae (36).

In the present work, the fungiform papillae of Group III exhibited distortion in shape together with wrinkling of the surface and ill-defined taste buds. These results are similar to those of another research which evaluated the effect of heavy metal (manganese) poisoning on the fungiform papillae of mice (37). The authors suggested that the swelling of taste buds in fungiform papillae would decrease function of these taste buds and probably inducing changes in food intake behavior as a consequence.

This is also in agreement with another analytical study in which; clinical evaluation of 70 subjects working in lead mines was done together with measurement of blood lead level (7). The study declared reduced taste perception and tongue burning in workers. The study attributed these changes mainly to the direct contact of the lead fumes with the oral mucosa.

When honey was given with lead in Group IV, a dose of 50 mg/kg of honey was given diluted in 1ml of distilled water (21). This low dilution was chosen to facilitate administration and to activate the antioxidant activity of honey. In a study to evaluate the effect of dilution of honey on its antioxidant activity, it proved that excessive increase in honey dilution lowered antioxidant capacity (38).

Simultaneous administration of honey with lead in the present study in Group IV appeared to ameliorate lead-induced histological alterations on the lingual papillae. In this group, the filiform papillae showed minimal changes when seen by scanning electron microscopy; the papillary density appeared increased compared to Group III, but apparently less than Group I.

The biochemical parameters were evaluated when honey was given with lead to rats using a dose of 10 mg/kg lead and 200 mg/kg honey both given for 7 weeks(16). The results of this study showed significant recovery in the biochemical alterations caused by lead toxicity upon consumption of honey. These findings suggested that honey may exert a protective role against lead toxicity. Three constituents of honey were suggested to have an antioxidant role namely; thiamin, riboflavin, and ascorbic acid. Thiamin inhibits or interferes with lead absorption due to complex formation between lead and thiamin. Ascorbic acid has also been reported to act as a detoxifying agent by forming poorly ionized, but soluble compounds with lead (16). This improvement in the biochemical analysis upon honey consumption is in agreement with the results of the current study where a marked improvement on the SEM level was observed.

Another research on the protective effect of honey on lead- induced oxidative stress in rat liver was conducted. Lead was given as (0.2%) in drinking water while 1.5 ml/kg honey was given orally for 4 weeks. The biochemical analysis showed that honey significantly increased antioxidant enzyme activity. The histopathological examination revealed that honey diminished the adverse effects of lead in rat liver, restoring normal liver architecture. The authors suggested that honey exerted its protective role through restoring enzymatic activity and through its antioxidant mechanism (39).

Honey is a potent source of iron, copper, and manganese. When these elements are combined they aid in hemoglobin synthesis. Honey is therefore a powerful weapon against anemia (40). Thus it may be suggested that the anti-anemic effect of honey may provide an alternative or an additional mechanism to explain the protective effect of honey against the lead-induced lingual atrophy.

It was reported that in cases of regeneration of atrophied lingual papillae; the fungiform papillae regenerate first followed by regeneration of filiform (36). This may explain the present findings where honey caused marked regeneration of the fungiform papillae. This was observed by SEM where the fungiform papillae of Group IV showed a close picture to that of Group I.

CONCLUSIONS AND RECOMMENDATIONS

The results of the current study showed that the occupational lead dose causes detrimental effects on lingual papillae of albino rats. Filiform papillae appeared to be the more sensitive to lead toxicity than fungiform papillae. Based on the results of this preliminary research, honey showed important protective role against lead-induced changes. Further experiments are needed to investigate the use of honey preparations with defined composition to assess the exact mechanism involved in this amelioration. Further research is needed to test whether the obtained results will also be achieved in human workers.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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