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Original Article

Physiological reaction of anxious patients taking sedative medications before and after periodontal surgery

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Sedation

Background/purpose: Clinicians use sedatives for anxiety patients at times in daily practice, but the direct influence of the medication on the wound healing of periodontal tissues is unknown. The aim of this study was to analyze the influence of the short-term administration of diazepam to patients with dental anxiety undergoing free gingival graft (FGG) procedures.
Materials and methods: A total of 51 FGG procedures in 39 patients were included. Twenty-six anxious patients medicated with 5 mg of diazepam from the night before surgery to 7 days after surgery served as the medication group, and the rest served as the control group. Direct examination, photographs and H_2O_2 were used to evaluate the healing of palatal wounds. Stress levels and sleep quality, and salivary melatonin levels were assessed.
Results: On Day 14, complete epithelization of the wounds was noted in 48.39% of the patients in the medication group and 35.29% of the patients in the control group. Regardless of whether they receive medication or not, groups with complete epithelialization by Day 14 had higher levels of preoperative melatonin than those without, with a *P* value of 0.02. The postoperative melatonin in the medication group tended to present higher levels than the control group.
Conclusion: Higher preoperative melatonin levels can accelerate wound healing. The short-term administration of the diazepam seemed to facilitate palatal wound healing by reducing

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stress and maintaining postoperative melatonin levels. This is the first time the relationships between sedatives, melatonin levels and palatal wound healing has been reported.
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Introduction

Mental health is associated with behaviors, physiological reactions, and disease manifestations. According to a study evaluating dental anxiety levels, periodontal surgery can cause significant anxiety.[1] It has also been pointed out that avoidance of dental treatment due to anxiety is common and may result in deterioration of oral and dental health.[2] Administration of oral sedatives is one of the solutions, and benzodiazepines (BZDs) are the most commonly prescribed medications to alleviate anxiety in dental procedures.[3] It has been reported that BZDs encourage stressed patients to receive the required treatment with positive outcomes.[4,5]

The earliest report to discuss the relationship between psychological status and periodontal disease is the study by Johnson and Engel.[6] They reported that military personnel were easily afflicted with necrotizing ulcerative gingivitis and emotional stress is one of the predisposing factors. Stress is considered a risk determinant of periodontal diseases.[7–10] Physiologically, the association between stress and periodontal disease can be attributed to two main mechanisms: activation of the hypothalamic—pituitary—adrenal (HPA) axis and the sympathetic nervous system.[11,12] High cortisol levels inhibit immune and inflammatory responses and alter the levels of blood glucose, growth factors, and cytokines.[13,14] Meanwhile, norepinephrine and epinephrine are released from the adrenal medulla under stress. These stress hormones exhibit an immunosuppressive effect.[7,12] A high cortisol level was reported to be correlated with the severity of periodontitis.[8,15] As immunity protects the wound from infection and regulates the repair process, stress may pose a negative impact on periodontal wound healing.[16]

Melatonin is an endocrine agent produced by the pineal gland and regulates sleeping in a circadian manner. Because melatonin provides, among others, antioxidant, anti-inflammatory, and antiviral functions, it has been suggested that melatonin may play a role in wound healing[17] and was correlated with the severity of periodontal diseases.[18] Because melatonin provides many functions related to wound healing,[19] some researchers think melatonin could be a potential treatment for patients with a sleep disorder or diabetes.[17,20]

Sufficient width of keratinized tissue (KT) is essential for the maintenance of periodontal and peri-implant health.[21,22] Among periodontal plastic surgeries, free gingival grafts (FGGs) are considered the gold standard solution for cases with insufficient KT.[23] Nevertheless, a major complication of the FGG procedure is the discomfort and morbidity in the donor site,[24] which often makes clinicians and patients hesitant to receive the required treatments.

To date, the impact of stress or sedative medications on periodontal wound healing has scarcely been investigated. The objective of this study was to investigate the influences of diazepam, one of the commonly used BZDs, on the healing of donor wounds of FGG. Salivary melatonin, a marker of sleep quality and a potential promoter of wound healing, was also assessed.

Materials and methods

Patients who required FGG treatment for implants or teeth without sufficient KT were recruited from Jan 2018 to Jul 2021 at the Department of Dentistry and they were enrolled after signing the informed consent form. Those who exhibited the following characteristics were excluded: 1) patients with major systemic diseases (including those with coagulation disorders, diabetes, respiratory diseases or immune-compromised patients) that potentially affect the healing of oral or periodontal wounds; 2) patients who are allergic to BZDs; 3) pregnant or lactating women; 4) women planning to become pregnant; 5) those taking any sedative medications, opioids, or tricyclic antidepressants for other illnesses; 6) current smokers; 7) patients with >20% plaque score[25] or >20% gingival bleeding score.[26]

Patients were given information, including the advantages and potential adverse effects of BZDs, to enable them to decide for themselves whether or not to take the medicine and recorded the times they took the medicine in the questionnaires. The examiner was unaware of their decisions after the final assessment of the palatal wound condition 2 weeks later. The patients were assigned to the control (without a diazepam prescription) and medication groups (with a diazepam prescription) based on their reports.

Preoperative protocol and surgical procedures

The workflow of the study is illustrated in Fig. 1. All patients received dental examination and full mouth scaling one week prior to the surgery. The following medications were provided for all patients: 500 mg amoxicillin (one hour before surgery and q8h for 5 days after surgery); 500 mg acetaminophen (one hour before surgery and q6h or as needed for 5 days after surgery); 400 mg ibuprofen (q6h or as needed for 3 days after surgery); and 5 mg diazepam (Dupin, China Chemical & Pharmaceutical Co., Ltd., Taipei, Taiwan), one hour before bedtime the night before surgery and for 7 days after surgery.

Preoperative questionnaires on the stress level and the sleep patterns were rated on a 4-point scale: 0 (no stress/good sleep), 1 (mild stress/slightly disturbed sleep), 2

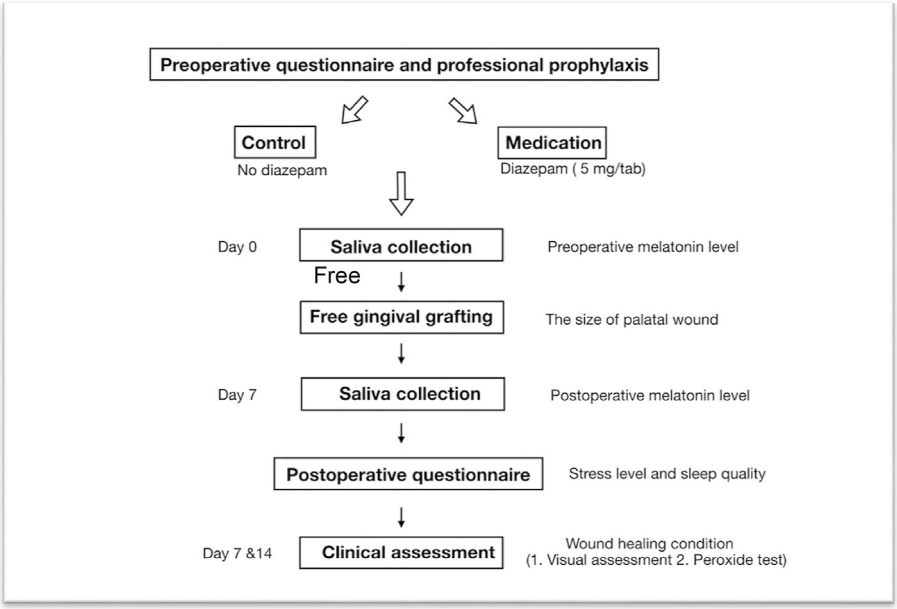

Figure 1 The workflow of the study.

(moderate stress/moderately disturbed), and 3 (severe stress/insomnia).[23] To assess the melatonin level, saliva was collected at 9 am using a cotton swab provided in a saliva sampling device (Salivette®, Sarstedt, Rommelsdorf, Germany).[27]

All clinical procedures were performed by an experienced certified periodontist (IPL). Local anesthesia of 1.8–3.6 ml 2% lidocaine and 1: 80,000 epinephrine were administered. The partial thickness flap at the recipient site was reflected, and the required size for augmentation was measured. A 1-mm thick FGG was harvested from the palate, and the dimensions of the palatal wound were recorded using a periodontal probe (CPU 15UNC, Hu-Friedy, Chicago, IL, USA). The FGG was subsequently adapted to the recipient bed and stabilized with 5-0 and 6-0 nylon using suspensory periosteal sutures. A dressing (COE-PAK™, GC, Chicago, IL, USA) was placed over the donor wound after hemostasis was achieved by cross sutures.

Postoperative protocol and clinical assessments

The patient was instructed not to brush the surgical areas but to rinse with 0.12% chlorhexidine gluconate twice a day for 2 weeks. The postoperative stress level, sleep patterns and compliance with medication were recorded in the questionnaires, and saliva was collected on Day 7. The wound dressing and all stitches at the donor site were then removed.

On Day 7, the healing of the palatal wounds was assessed visually to avoid interfering with the healing process. On Day 14, the healing of the palatal wounds was assessed with the hydrogen peroxide (H_2O_2) test. The wound was rated as complete wound healing if there was no oxygen liberation and was recorded as a dichotomous variable (yes/no) (Fig. 2).[28] The level of healing potential was classified: grade I: complete epithelialization; grade II: two-thirds of the wound with epithelialization; grade III: one-third of the wound with epithelialization; grade IV: no re-epithelialization.[28]

Measurement of salivary melatonin level

All saliva samples were centrifuged at 3000 rpm for 5 min immediately after collection and then frozen at $-80\ ^{\circ}C$ in aliquots. Samples were thawed and analyzed within 6 months. The level of salivary melatonin was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Salimetrics™ Assay #1-13402, Carlsbad, CA, USA). All samples were assessed two times, and the mean values were documented in the first batch.

Statistical analysis

Data were tested for normality using the Shapiro–Wilk test. The palatal wound sizes and salivary melatonin levels were compared using the Wilcoxon rank-sum test (Table 2). Correlations between the melatonin levels and the healing condition were analyzed using the Wilcoxon rank-sum test (Table 3). The cutoff value to evaluate the impact of medication on wounds of different sizes was set at 150 mm^2, the median wound size (Table 4). A power analysis was performed, which showed that 25 participants in the medication group and 15 in the control group would provide 83.4% power. All analyses were performed using SAS statistical software (SAS System for Windows, version 9.4; SAS Institute, Cary, NC, USA). All *P* values reported were 2-sided, and the significance level was set as <0.05.

Results

A total of 51 palatal wounds were assessed in 39 patients. Periodontal parameters were within the normal range (Table 1). However, 3 patients delayed their follow-up

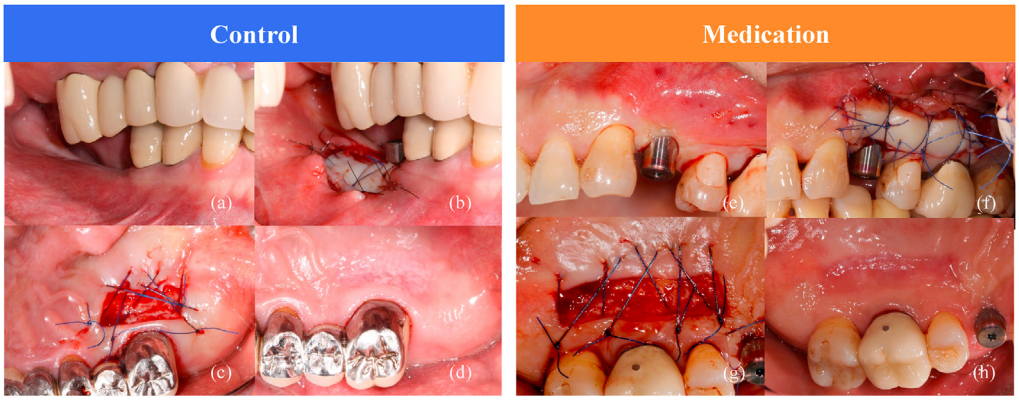

Figure 2 Clinical photographs of the control and medication groups. (a, e) Recipient site. (b, f) Application of a free gingival graft. (c, g) Palatal wound of 1 mm in depth. (d) Incomplete wound epithelialization with the foaming reaction by H_2O_2 test at 2 weeks. (h) Complete wound epithelialization at 2 weeks.

visits; therefore, their data on Day 14 are missing. Additionally, 6 saliva samples were excluded because sufficient saliva had not been collected for the analysis. There was no significant difference in the size of initial palatal wounds, stress levels and sleeping patterns between control and medication groups (Table 2)., On Day 7, none of the wounds were completely healed regardless of treatment, and most wounds were covered with newly formed granulation tissues, fibrin clots and sloughed tissues, which made it difficult to assess the extent of healing. On Day 14, 35.29% of the wounds in the control group and 48.39% of the wounds in the medication group were completely healed (Table 2). To assess the wound healing potential, 70.59% of the wounds healed well in the control group, whereas 90.32% of the wounds in the medication group presented a good level of healing.

In the postoperative questionnaires, there was no statistically significant difference in the stress level or sleeping patterns between the medication and control groups (Table 2). However, in patients with larger wounds (>150 mm^2), the stress for future surgery was elevated in the control group (from 0.80 ± 0.45 to 1.00 ± 0.71) but was reduced in the medication group (from 0.95 ± 0.58 to 0.77 ± 0.53) (Table 4).

High preoperative melatonin levels showed a higher percentage of complete wound healing whether or not in the test or control groups, with a *P* value of 0.0024 (Table 3). No statistically significant difference in the preoperative melatonin levels was noted between the medication and control groups. The patients in the control group tended to present lower postoperative melatonin levels than those in the medication group (Table 2).

Discussion

Numerous efforts have aimed to promote healing and reduce the discomfort and/or morbidity of donor wounds from FGG

Table 1 Demographic characteristics of all participants.

	Control		Patient		*P* value
	(N = 13)		(N = 26)		
Age, Mean ± SD	54.08	(8.87)	54.65	(7.87)	0.7998
Gender, (%)					0.6452
Male	7	(53.85)	16	(61.54)	
Female	6	(46.15)	10	(38.46)	
Mean PPD (mm)					0.5203
Mean ± SD	2.77	(0.33)	2.85	(0.37)	
Median (IQR)	2.78	(2.55,2.93)	2.74	(2.68,2.98)	
Mean recession (mm)					0.2806
Mean ± SD	1.28	(0.73)	1.60	(0.80)	
median (IQR)	0.97	(0.70,2.04)	1.65	(0.94,2.08)	
Mean CAL (mm)					0.5529
Mean ± SD	4.03	(0.64)	4.45	(0.96)	
Median (IQR)	3.96	(3.82,4.61)	4.39	(3.65,5.14)	
Plaque score (%), Mean ± SD	14.73	(3.57)	12.74	(4.46)	0.2492
Bleeding score (%), Mean ± SD	9.95	(4.96)	9.38	(5.30)	0.7064

Abbreviations: SD (Standard deviation); IQR (Interquartile range); PPD (Probing pocket depth); CAL (Clinical attachment level). Fisher's exact test; Wilcoxon rank sum test.

Table 2 Wound size, healing condition, stress level, sleep patterns, and melatonin level.

	Control		Patient		*P* value
	(N = 18)		(N = 33)		
Palatal wound size (mm^2), Mean ± SD	145.60	(35.71)	162.21	(37.14)	0.1574
Wound condition (1st week), (%)					
Healed	0	(0)	0	(0)	
Unhealed	17	(100)	31	(100)	
Wound condition (2 nd week), (%)					0.38183
Healed	6	**(35.29)**	15	**(48.39)**	
Unhealed	11	(64.71)	16	(51.61)	
Wound healing potential, (%)					
Good	12	**(70.59)**	28	**(90.32)**	
Grade I	6		15		
Grade II	6		13		
Poor	5	(29.41)	3	(9.68)	
Grade III	5		3		
Grade IV	0		0		
Stress level, (%)					
Before operation, Mean ± SD	1.00	(0.67)	0.94	(0.50)	0.7842
For the future same operation, Mean ± SD	0.90	(0.57)	0.76	(0.56)	0.4926
Sleeping patterns, (%)					
Preoperative, Mean ± SD	0.60	(0.52)	0.70	(0.53)	0.6452
Postoperative, Mean ± SD	0.80	(0.42)	0.91	(0.58)	0.6305
Melatonin (pg/ml)					
Preoperative					0.8662
Mean ± SD	49.04	(25.33)	55.46	(35.24)	
Median (IQR)	44.03	(28.40,67.60)	41.53	(29.74,83.30)	
Postoperative					0.1148
Mean ± SD	**33.32**	(7.73)	**46.77**	(28.03)	
Median (IQR)	**35.00**	(26.63,40.07)	**38.46**	(28.86,47.30)	

Abbreviations: SD (Standard deviation); IQR (Interquartile range).
Fisher's exact test; Wilcoxon rank sum test.
The values that are significant in this table have been in bold format.

procedures,[28–31] the impacts of stress on this aspect have rarely been discussed. Diazepam, a common BZD for patients with anxiety or insomnia, is often offered as a stress reduction strategy to encourage patients to receive treatment.[4] To avoid drug addiction, tolerance, dependence, or withdrawal symptoms, BZDs are recommended for short-term use (< 4 weeks).[32] In the present study, diazepam was administered only for 8 days.

Table 3 Correlation between wound condition and melatonin levels.

Wound condition	Preoperative melatonin level			Postoperative melatonin level		
			P-value			P-value
Unhealed			**0.0024**[a]			0.0771
Mean ± SD	40.00	20.37		36.62	16.48	
Median (IQR)	35.18	17.63		35.24	16.32	
Healed						
Mean ± SD	69.77	37.06		49.91	29.88	
Median (IQR)	73.95	59.91		40.29	14.65	

Abbreviations: SD (Standard deviation); IQR (Interquartile range).
Wilcoxon rank sum test.
The values that are significant in this table have been in bold format.
[a] $p < .05$.

Wound healing begins with blood clot accumulation and progresses through the elimination of necrotized tissues, formation of granulation tissue, approximation of epithelium and connective tissue, and finally tissue maturation.[33] Complete epithelialization of donor palatal wounds usually takes 2–4 weeks.[34] In the present study, the percentage of patients in the control group who achieved complete wound healing by Day 14 was 35.29%, which was higher than that in the study by Yaghobee et al.[28] and higher than that of smokers in the experiment by Silva et al.[35] The variable results can be attributed to the differences in the study design and initial size of the palatal wounds.

The major complications with the FGG technique before the completion of epithelialization include acute pain, excessive hemorrhaging and bone exposure.[24] Since donor site morbidity is a major complication of the FGG procedure,[24] many strategies have been attempted and faster wound healing of palatal wounds, as well as reduced postoperative pain, were reported with these interventions.[29–31] Interestingly, the present study showed that 48.39% of the patients medicated with diazepam demonstrated complete epithelialization of palatal wounds,

Table 4 Comparison of wound condition, stress level, and sleeping patterns between patients with small and large wound sizes.

Palatal wound size (mm^2)	Control (N = 18)					Medication (N = 33)					Control vs. Medication of *P* value	
	Small (N = 11)		Large (> 150) (N = 7)		*P* value	Small (N = 11)		Large (> 150) (N = 22)		*P* value	Small (n = 22)	Large (> 150) (n = 29)
Wound condition, (%)					0.1618					1.0000	0.3498	1.0000
Healed	8	(80.00)	3	(42.86)		5	(50.00)	11	(52.38)			
Unhealed	2	(20.00)	4	(57.14)		5	(50.00)	10	(47.62)			
Wound healing potential, (%)					1.0000					0.5328	0.2105	0.5737
Good	7	(70.00)	5	(71.43)		10	(100.00)	18	(85.71)			
Poor	3	(30.00)	2	(28.57)		0	(0.00)	3	(14.29)			
Stress level, (%)												
Before operation					0.4065					0.8584	0.3321	0.6133
Mean ± SD	1.20	(0.84)	0.80	(0.45)		0.91	(0.30)	0.95	(0.58)			
Median (IQR)	1.00	(1.00, 2.00)	1.00	(1.00, 1.00)		1.00	(1.00, 1.00)	1.00	(1.00, 1.00)			
For the future same operation					0.6985					0.7864	0.7906	0.4743
Mean ± SD	0.80	(0.45)	1.00	(0.71)		0.73	(0.65)	0.77	(0.53)			
Median (IQR)	1.00	(1.00, 1.00)	1.00	(1.00, 1.00)		1.00	(0.00, 1.00)	1.00	(0.00, 1.00)			
Sleeping patterns, (%)												
Preoperative					1.0000					0.2654	0.8953	0.5444
Mean ± SD	0.60	(0.55)	0.60	(0.55)		0.55	(0.52)	0.77	(0.53)			
Median (IQR)	1.00	(0.00, 1.00)	1.00	(0.00, 1.00)		1.00	(0.00, 1.00)	1.00	(0.00, 1.00)			
Postoperative					1.0000					0.9817	0.8435	0.7140
Mean ± SD	0.80	(0.45)	0.80	(0.45)		0.91	(0.70)	0.91	(0.53)			
Median (IQR)	1.00	(1.00, 1.00)	1.00	(1.00, 1.00)		1.00	(0.00, 1.00)	1.00	(1.00, 1.00)			

Abbreviations: SD (Standard deviation); IQR (Interquartile range).
Fisher's exact test; Wilcoxon rank sum test.

whereas only 35.29% of those in the control group presented complete wound healing on Day 14. To further evaluate the healing potential by assessing the ratio of healed/total wound areas, a much higher proportion of well-healing wounds was observed in the medication group than in the control group (90.32% and 70.59%, respectively). It is speculated that the sedative, diazepam, may trigger some physiological reactions to facilitate the healing of palatal wounds following FGG surgery.

The correlation between melatonin and wound healing, as well as stress levels, has been pointed out. Melatonin regulates a series of signaling pathways, such as the inhibition of advanced glycation end product (AGE)-mediated cellular dysfunction and apoptosis, the reduction of inflammatory cytokines and direct scavenging of reactive oxygen species (ROS). Because of its antioxidative and anti-inflammatory properties and capability to stimulate type I collagen formation,[22,36] melatonin has been shown to facilitate wound healing. Moreover, it has been reported that the melatonin level is reduced in stressful situations, which may result from the inhibitory effect of corticosterone or the consumption of melatonin to reverse the adverse effects of stress.[37] Since the melatonin levels fluctuate over the course of a day, we collected all samples at the same time, as described in the study by Cutando et al.[18] One of the important finding in this study is that higher melatonin levels appeared to be associated with accelerated wound healing regardless of medication use (Table 3), which is in accordance with previous studies.[17,19,38] Another is that the majority of the patients in the medication group tended to present higher melatonin levels postoperatively than those in the control group. It is assumed that the levels of postoperative melatonin might have been maintained as a result of stress reduction and/or high-quality sleep due to diazepam use. This result may also account for a higher percentage of complete wound healing and an even higher result for good healing potential in the medication group (Table 2).

In this study, patients with dental anxiety decided to take the medication. According to the patient-reported outcomes, there was no significant difference in stress levels before surgical operations between the control and medication groups. This result would indicate that the sedative medication helped reduce the stress levels for the worried patients to some extent (Table 2). When just focusing on large wounds, participants in the medication group presented lower stress levels for the prospect of receiving the same operation in the future than those in the control group, underscoring the possible beneficial influences of the sedative on stress reduction before surgery (Table 4).

The impact of diazepam may be underrated in the present study because several patients on diazepam had suffered from pre-existing insomnia and had a history of compromised wound healing in previous operations. Since each patient had his or her unique disposition, lifestyle, socioeconomic status, coping abilities and social network, the influence of diazepam on stress or anxiety reduction could not be objectively assessed.[7,9] Last but not least, the pharmacokinetics of the orally administered drug vary between individuals.[4] Therefore, future investigations are required to validate its usage and find the most effective dose, and routes of administration with more objective assessment methods.

In conclusion, this pilot study revealed that many patients were concerned about soft tissue grafting and the administration of sedatives would help them to receive the required treatments and reduce stress levels. It is likely that the sedative may be able to maintain postoperative melatonin levels, resulting in the acceleration of the healing of palatal donor wounds. Therefore, sedatives can be considered for use in dental practice, especially for stressful surgical treatments. Given the limitations of the study, additional investigations are needed to elucidate the mechanisms and conclusively determine effects of the sedative medications on periodontal wound healing.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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