Provocative challenges to help diagnose and monitor asthma: exercise, methacholine, adenosine, and mannitol

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Purpose of review
To review bronchial provocations tests used in the measurement of bronchial hyperresponsiveness to help in the diagnosis of asthma.
Recent findings
The bronchial provocations tests reviewed include exercise, methacholine, AMP and mannitol, with reference to methodology and monitoring of treatment.
Summary
Methacholine is used for identifying bronchial hyperresponsiveness and to guide treatment. Exercise is used as a bronchial provocation test because demonstrating prevention of exercise-induced asthma is an indication for use of a drug. Both of these tests are being used to study tolerance to β_2 agonists. There is increasing use of eucapnic voluntary hyperpnea as a surrogate bronchial provocation test for exercise to identify exercise-induced asthma, particularly in athletes. For methacholine and AMP there is concern about the different breathing patterns used to inhale these aerosols and the impact they have on the cutoff point for identifying bronchial hyperresponsiveness. A new test that uses a kit containing prepacked capsules of different doses of mannitol and a delivery device is discussed. There is increasing interest in using tests that act indirectly by release of mediators because the bronchial hyperresponsiveness itself is an indicator of the presence of inflammation. Since treatment of inflammation leads to loss of bronchial hyperresponsiveness to indirect stimuli, these tests are well suited to identify success of treatment.

Keywords
adenosine, exercise, mannitol, methacholine

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Introduction

While bronchial provocation tests (BPTs) using stimuli that act directly on smooth muscle receptors continue to be commonly used, the advantages of using indirect stimuli that act by release of mediators are increasingly being recognized (Table 1) [1]. One of the advantages of using indirect stimuli to diagnose the bronchial hyperresponsiveness (BHR) of asthma is that all the common stimuli that provoke an attack of asthma in daily life, for example allergens, cold air, exercise, sulfur dioxide, and fog, act indirectly to cause airways to narrow. These same indirect stimuli can also provoke cough so this feature has the potential to be used to determine if a patient's cough is associated with airway narrowing during challenge. Importantly, the success of a treatment can be gauged by how well it protects the asthma patient against attacks from exposure to these common stimuli. For example, well treated asthma patients lose their sensitivity to indirect stimuli in response to an appropriate dose of inhaled corticosteroids [2], and remission of exercise-induced asthma (EIA) is a good example of this.

Exercise

Exercise is a commonly used BPT, particularly in children. An advance in methodology is suggested by the improved sensitivity to identify BHR in 3–6 year olds by measuring changes in forced expiratory volume (FEV) in 0.5 s ($FEV_{0.5}$) rather than FEV_1 in response to exercise. A value of at least 13% was used as the cutoff point based on the FEV_1 data for EIA [3••]. This seems a reasonable approach and the cutoff point for $FEV_{0.5}$ could be checked retrospectively in the healthy population for spirometry results stored on computers. In contrast, and perhaps unexpectedly, measuring the changes in mid-expiratory flow rates was not shown to improve sensitivity to identify EIA in athletes using eucapnic voluntary hyperpnea (EVH), even though this test is an excellent surrogate for exercise [4••,5].

Table 1 Advantages and disadvantages of bronchial provocation tests

Challenge	Advantages	Disadvantages
Exercise	The real stimulus that produces the symptoms and signs of EIA High positive predictive value for identifying asthma Common trigger for an asthma attack Many mediators involved in EIA including histamine, prostaglandins and leukotrienes Useful to provide an indication for a drug used in the treatment of asthma	Ergometers are expensive and space occupying Inspired air needs to be 'dry' Requires 6–8 min of vigorous exercise at 85–95% HR_{max} so there are limitations in testing everyone Sensitivity to identify EIA in the laboratory setting can be low, particularly in elite athletes Single 'optimal' stimulus so severe responses (>50%) can occur A 20% fall for the response is needed for good repeatability Several personnel required for testing the patient
Eucapnic voluntary hyperpnea	High sensitivity to identify EIA Protocol and inspired air conditions can be adjusted to simulate conditions of a specific sport, e.g. rowing, skating Negative test highly likely to exclude EIA Equipment less expensive compared with exercise	Special gas mixture needed (~5% CO_2, 21% O_2, balance N_2) or special equipment for mixing gases Expensive to have a commercially prepared gas mixture Less sensitive when test duration is less than 6 min yet a 6 min protocol can provoke a severe response (>50%)
Methacholine	Sensitive test to identify BHR in a doctor-referred population Dose–response curve obtained Predictor of risk for developing asthma later in life or sign of previous asthma Negative test in symptomatic patients is useful to exclude asthma but does not exclude EIA FDA approved formulation available	Response is only being tested to one mediator or agonist Response in same person varies with the breathing pattern (tidal breathing or big breath) used to deliver the aerosol Different cutoff points used to identify hyperresponsivness A negative test does not exclude EIA Positive test not specific for asthma as people with airflow limitation or airway remodelling are also positive Positive test occurs with airway injury Solutions need to be prepared and nebulizers calibrated Not widely approved by regulatory agencies outside USA
AMP	Acts by mast cell release of mediators More sensitive for assessing response to corticosteroids than methacholine and relates better to other surrogate markers of inflammation Dose–response curve obtained	Wet aerosol by nebulizers delivering different doses Solutions need to be prepared and may be unstable Different cutoff points used to identify hyperresponsivness Response in same person varies with the breathing patterns (tidal breathing or big breath) used to deliver the aerosol Not approved by any regulatory authority for inhalation
Mannitol	Available as a convenient and standardized test kit with prefilled dry powder capsules and single use dry powder inhaler device Standard operating procedure for dose and delivery Approved by regulatory authorities in Europe and Australia Dose–response curve obtained Positive test predicts active asthma and potential for EIA More than one mediator involved, e.g. prostaglandins, leukotrienes, histamine Negative test in an asthmatic = good control of asthma Response–dose ratio may be used to monitor an intervention or back titration of steroid dose	May not be as sensitive as direct challenges to identify BHR in some population groups Some cough during challenge

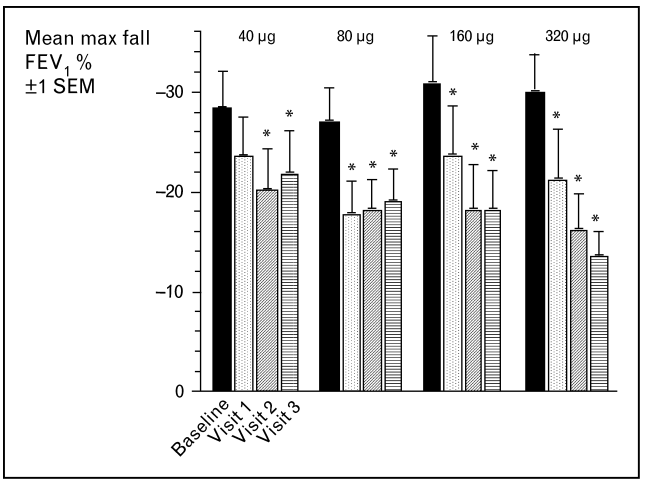
EIA, exercise-induced asthma; HR, heart rate; BHR, bronchial hyperresponsiveness; FDA, US Food and Drug Administration.

A study of British Olympic athletes using EVH and changes in FEV_1 observed that EIA was both under and overdiagnosed [6]. This overdiagnosis of EIA in athletes is confirmed by the report that when the International Olympic Committee Medical Commission required athletes to provide objective evidence of their asthma or EIA at the Summer Games in Athens there was a 26% reduction in the number of athletes requesting to use a β_2 agonist compared with the Games in Sydney [7••].

Exercise is used as a BPT because prevention of EIA provides an indication for use of a drug. The study of Pearlman *et al.* [8••] is a good example of this. They report that the protective effect of a single dose of montelukast is evident by 2 h and persisted throughout 24 h. This finding is important because this drug is expensive and many people only exercise once a week. Another study on montelukast demonstrated, for the first time in children, that no tolerance to the protective effects develops with daily use over 4 weeks [9].

The study by Subbarao *et al.* [10••] reports the effects of doubling doses of the inhaled corticosteroid (ICS) ciclesonide (40, 80, and 160, 320 μg/day) on the response to exercise during 3 weeks of treatment. The results showed benefit within 1 week at doses of 80 μg and above but greater benefit from receiving 320 μg once daily for 3 weeks (Fig. 1). The young adults in this study had mild

Figure 1 Maximum fall in forced expiratory volume in 1 s (FEV_1) expressed as a percentage of the preexercise value following exercise in young adults (aged 14–27 years) with a baseline FEV_1 of 92.3% predicted



Mean (SEM) values are for baseline, and after 1, 2 and 3 weeks of treatment with different doses of ciclesonide in two groups. One group took 40 and 160 μg whilst the other took 80 and 320 μg. The maximum % fall in FEV_1 decreased after 1 week in all but the 40 μg group and in all groups on subsequent weeks. The rate of improvement with the low doses (40 and 80 μg) plateaued after 1 week of therapy while continuing to improve with the high doses (160 and 320 μg). Reproduced from the data by Subbarao *et al.* [10••].

symptoms and were only taking a β_2 agonist. These findings remind us that while several weeks of treatment with low doses of ICSs can eliminate symptoms and normalize spirometry, higher doses of ICSs for a longer period are usually required to reduce the fall in FEV_1 to the normal range after exercise ($\leq$10% from baseline) [11]. Thus the potential for exercise to provoke an attack of asthma will remain for the majority of patients when they are prescribed short-acting β_2 agonists (SABAs) or low doses of ICSs alone or low doses of ICSs in combination with a long-acting β_2 agonist (LABA) for their asthma. This is evident by the widespread use of taking extra doses of β_2 agonist immediately before exercise.

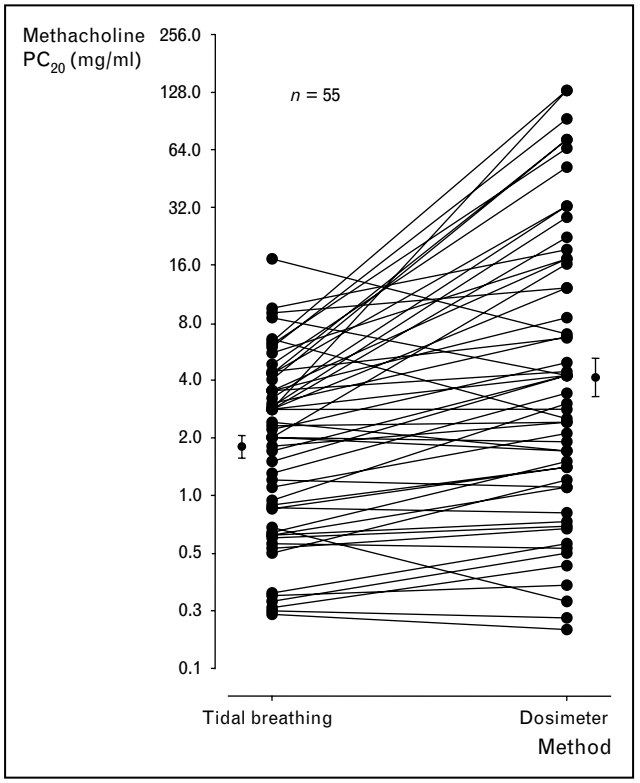
There has been robust discussion in the last 18 months given to the daily use of β_2 agonists and the potential for development of tolerance, particularly with respect to EIA and other mast cell-mediated stimuli [12•,13]. While the superiority of treatment with a combination of fluticasone and salmeterol (250/50 μg twice daily) compared with fluticasone alone (250 μg twice daily) was reported for protection against EIA, the efficacy (fall in $FEV_1 < 10\%$ of baseline) of the combination was still only 67% after 4 weeks when exercise was performed 1 h after dosing [14]. In a study in asthma patients taking low doses of steroids by Storms *et al.* [15], the development of tolerance to a β_2 agonist was demonstrated by a slower rate of recovery of FEV_1 following exercise during daily treatment with salmeterol but not montelukast. The clinical relevance of tolerance is that the expected benefits from a β_2 agonist on prevention and rapid reversal of attacks of asthma, particularly by exercise and allergens, may be compromised when these drugs are used everyday, even in the presence of inhaled steroids [16,17••].

We can now add epithelial injury to atopy, immunoglobulin E (IgE), and total eosinophil count and increased concentration of leukotrienes as part of the phenotype of EIA [18–21]. Thus an association between the epithelial cell number in sputum and EIA severity is described [18]. The same investigators [21] reported an increase in the gel-forming mucin MUC5AC from goblet cells in sputum 30 min after exercise. In the same paper they report an association between the levels of cysteinyl leukotrienes (cystLTs) and neurokinin A in induced sputum after exercise. This extends their earlier findings of an increase in concentration of inflammatory mediators in sputum in asthma patients with EIA that was inhibited by two doses of loratadine and montelukast taken 12 and 36 h before exercise [18,19]. Those findings are important because they indicate that the antagonists not only blocked the effect at the receptor but they also switched off the release of the mediators. Another novel observation is the study in asthma patients [22••] showing that fish oil supplements reduce EIA severity and the release of the mediators relevant to EIA.

Methacholine and AMP

A current issue under discussion is the method of administration of methacholine and AMP. Both these BPTs are given as wet aerosols. One issue relates to differences in dose delivered by different nebulizers and another relates to the breathing pattern used to inhale the aerosol. When methacholine is inhaled using deep inspiration to total lung capacity, as with the dosimeter method, bronchoprotection can occur and a person may be negative, yet have a positive response when the tidal breathing technique is used [23,24••]. A recent review from experienced investigators concluded that there is a 'marked lack of comparability' between the two methods commonly used to administer methacholine, particularly in patients with mild BHR [provoking concentration to cause a 20% fall in FEV_1 (PC_{20}) > 2 mg/ml] (Fig. 2) [24••]. This is of concern because it is these patients who are most likely to be referred for a challenge. This difference in sensitivity between two methods of aerosol delivery for methacholine has been confirmed by others [25]. The study by Prieto *et al.* [25], however, showed no difference in reactivity (slope) or the plateau in response to methacholine between the two methods. They suggested that it is the difference in delivered dose between the two methods (90 versus 45 μl at each concentration) that accounts for the difference in sensitivity rather than the bronchoprotective effect provided by a deep inspiration. The same group [26••] has reported similar discrepancies in values for PC_{20} when delivering AMP by the two different methods.

Figure 2 Provoking concentration to cause a 20% fall in forced expiratory volume in 1 s (PC_{20}) after methacholine in response to tidal breathing method (left) and dosimeter method (right) expressed in a log scale



The geometric mean tidal breathing PC_{20} was 1.8 mg/ml and with the dosimeter was 4.1 mg/ml ($P < 0.0001$). Those with a PC_{20} above 2 mg/ml on the tidal breathing method are more likely to be classified as unresponsive (>16 mg/ml) on the dosimeter method. Reproduced with permission from Cockcroft and Davis [24••].

Deep inspiration is well known to have a bronchoprotective effect and it is greatest in those who have mild asthma. Nonetheless, method of delivery may explain some unexpected negative findings in people with good lung function but symptoms of asthma and positive to other tests like exercise, EVH or mannitol [27]. These methodological issues do not present a problem for research studies on mechanisms in which the same technique and the same nebulizers are used by the same trained staff. For guidelines recommending use of these aerosols in diagnosis and treatment, however, it becomes complex if the breathing pattern and delivery devices need to be specified to identify a suitable cutoff point for BHR [28,29]. To add to this problem is the need to calibrate nebulizers regularly if dose rather than concentration is reported, as commonly is the case outside North America [30,31]. Finally, it is also difficult to determine equivalence in cutoff points for dose and concentration [29]. Is 1 mg or 5 μM given by a dosimeter really equivalent to 10 mg/ml by tidal breathing [7••]?

Methacholine is also used to identify tolerance to β_2 agonists. As with recovery from exercise, when LABAs are being used regularly there is a subsensitivity to recovery with a SABA following acute bronchoconstriction with methacholine in adults [32]. This has now been reported for the first time in children, and all were taking ICSs [33••]. After 2 weeks of treatment with the LABA formoterol, the time for recovery in response to a SABA from bronchoconstriction induced by methacholine was over 20 min compared with 10 min after placebo [33••]. It would seem a small but valuable step forward if commonly used protocols for BPTs could be extended to include time for recovery in order to identify tolerance to β_2 agonists.

A study by Hanxiang *et al.* [34••] is important because it cautions readers to reconsider the definition of 'total control', using symptoms and lung function as a guide. These authors suggest that measurements of inflammation and BHR also need to be included in assessing control of asthma. They found 65/76 patients with 'total control', taking inhaled steroids alone, had BHR to methacholine ($PC_{20} < 8$ mg/ml) and evidence of airway inflammation in induced sputum, as measured by increased percentage of eosinophils [34••].

A study in children [35••] did not find a change in number of symptom-free days when BHR to methacholine and symptom strategy was used for guiding dose of ICS compared with symptom strategy alone. The finding that a subgroup (BHR, but low symptom scores) of children were at risk of a decline in lung function over the 2 years is very important. Thus there was a 6% difference ($P < 0.017$) in FEV_1% predicted between the two strategy arms, for this subgroup, with those being in the BHR strategy achieving a higher FEV_1. This suggests that measurements of BHR should be included in studies of children if good lung function is to be maintained.

The anti-inflammatory effects of ICSs on AMP challenge were demonstrated by Wilson *et al.* [36••] in response to 4 weeks of treatment with 160 μg (once daily) of ciclesonide. There was a seven-fold increase in mean values for PC_{20} to AMP (SEM) from 17 (8) to 140 (63) mg/ml and this was associated with a reduction of eosinophils in induced sputum. In contrast, Green *et al.* [37••] showed, after 4 weeks of treatment with twice the equivalent dose of budesonide (800 μg per day), only a 0.4 (−0.2–1.0) doubling dose reduction in PC_{20} to methacholine from a baseline of 0.39 ± 0.1 mg/ml associated with a 1.6 (1.2–2.2)-fold reduction in sputum eosinophils. Thus responsiveness to AMP appears to provide a better reflection of changes in inflammatory markers than responsiveness to methacholine.

Young children have been included in studies comparing responses to both methacholine and AMP [38,39]. These studies have shown that AMP responsiveness, like exercise, is more likely to be associated with atopy, IgE, and inflammatory markers such as peripheral blood eosinophils compared with methacholine [38]. In studies from different investigators, AMP responsiveness in children was predicted by peripheral blood eosinophilia (odds ratio 5.14, $P = 0.025$), but not atopy, in children aged 3–6 years [40], whereas in children aged 7–16 years grass pollen sensitization (odds ratio 5.65, $P < 0.003$) was an independent predictor [39].

Mannitol

Mannitol, given by inhalation as a dry powder (Aridol/Osmohale, Pharmaxis Ltd, Frenchs Forest, New South Wales, Australia) became the first BPT to be approved by regulatory authorities in the European Community (2007) and in Australia (2006) following a phase 3 trial of safety and efficacy [41]. The mannitol test comes in a kit with prepacked capsules containing different doses of powder (5, 10, 20 and 40 mg) and a delivery device. This kit provides for the first time a standard operating procedure for dosing and delivering a provoking agent. This feature should make the mannitol test equally attractive to individual practitioners, laboratories and for multicentre trials and epidemiological studies across geographical boundaries.

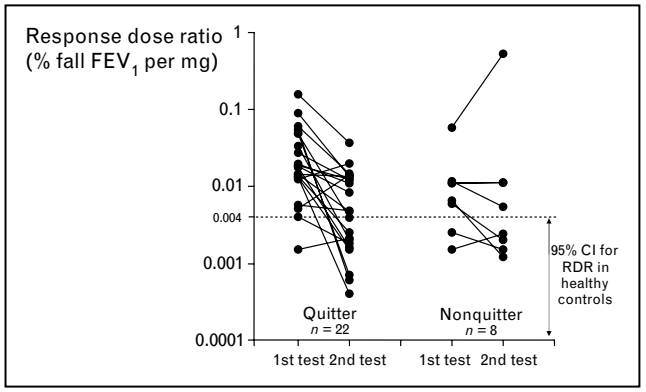
The hyperosmolar aerosol BPT arose from the proposal that exercise provoked bronchoconstriction by causing a transient increase in osmolarity of the airway surface and consequent release of mast cell mediators [42]. The mannitol test was developed to overcome the technical issues associated with carrying out other BPTs. Inhaling mannitol to increase osmolarity of the airway surface overcame the problems of need to perform vigorous exercise breathing dry air. The dry powder device used for delivering the various doses of mannitol overcame the issues in making up solutions and generating and delivering wet aerosols with different nebulizers. In known asthma patients there is a good relationship between response to mannitol and the response to AMP [43], hyperosmolar saline [44], exercise [45], and methacholine [44]. Importantly the drugs used in the treatment of asthma modify the response to mannitol, so it has the potential to be used to monitor treatment [2].

Like exercise and AMP, mannitol acts indirectly to cause contraction of smooth muscle by release of inflammatory mediators such as leukotriene E_4 and the mast cell specific mediator prostaglandin D_2 [46]. The new finding is that the increase in urinary excretion of the prostaglandin D_2 metabolite, 9α,11β-prostaglandin (PG) F_2, but not leukotriene E_4, was blocked by inhaling either 40 mg of sodium cromoglycate or 24 μg of formoterol immediately before challenge in asthma patients. This is the first report *in vivo* and provides evidence for the long held view from in-vitro studies that these drugs prevent bronchoconstriction to indirect stimuli by inhibiting release of mediators from mast cells [1,47••].

That BHR to mannitol is associated with currently active asthma was supported by the findings of Porsbjerg *et al.* [48••]. The response to mannitol was investigated in 16 patients with airway hyperresponsiveness to methacholine (GM PD_{20} 2.25 μmol, 95% confidence interval 2.19, 5.29) but no respiratory symptoms for 12 months and no diagnosis of asthma. A positive response to mannitol was documented in only one patient, a current smoker with rhinitis identified as episodes of sneezing [48••].

Responsiveness to mannitol appears to be dependent on the presence of airway inflammation and the findings of Stolz *et al.* [49••] in smokers are of interest. They studied responses to mannitol in asymptomatic smokers with normal spirometry attending a stop smoking clinic. They found the response dose ratio, an index of reactivity, to mannitol was higher than in nonsmoking healthy controls and was significantly reduced ($P < 0.0001$) after stopping

Figure 3 Response dose ratio – the maximum % fall in forced expiratory volume in 1 s (FEV_1) from baseline after being divided by the cumulative dose delivered in milligrams – is used as a measure of reactivity to mannitol and permits analysis of data from all patients



There was a significant reduction in reactivity to mannitol after stopping smoking ($P < 0.0001$). The 95% confidence interval (CI) for response dose ratio (RDR) in healthy individuals is taken from the data by Brannan *et al.* [41]. Reproduced from Stolz *et al.* [49••].

smoking (Fig. 3). This reduction was not related to improved airway caliber, as the FEV_1 was marginally reduced after stopping smoking. The authors suggested this change may reflect a reduction in airway inflammation after stopping smoking [49••]. An unexpected finding in the study was a higher rate of success at quitting smoking in those who had a 15% or more fall in FEV_1 to mannitol (PD_{15}) [49••]. The potential for mannitol to be used to identify asthma in occupations such as firefighters is interesting in this respect [50•].

Conclusion

The research community is trying to optimize the use of the two types of BPTs available to identify BHR with a view to aiding the diagnosis of asthma and to identify tolerance to β_2 agonists. The two types are based on whether the stimulus acts directly on smooth muscle, as with methacholine, or indirectly by the release of mediators. The current concern about methacholine is that its advantage in providing a high negative predictive value can be lost if the aerosol is administered using deep inspiration to total lung capacity. The stimuli that act indirectly via release of mediators such as exercise remain popular to assess both acute and chronic effects of drugs used in the treatment of asthma. Surrogates of the exercise stimulus have been developed and include eucapnic voluntary hyperpnea and mannitol. Mannitol is a new commercially available BPT and its delivery by inhalation as a dry powder is standardized. Current studies suggest that responsiveness to stimuli that act indirectly are better predicted by inflammatory markers than the responsiveness to directly acting stimuli. The potential use of these indirect stimuli to identify BHR is reported in children, athletes, smokers and for occupational assessment. Since treatment of airway inflammation or avoidance of contributory factors can potentially normalize the airway response to an indirect stimulus, these BPTs are being increasingly used to identify success of treatment.

Acknowledgements

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Dr Anderson purchased shares on the open market and does not hold options. She may benefit from distribution of royalties in the future.

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- •• of outstanding interest

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