

Diagnostic properties of inhaled mannitol in the diagnosis of asthma: A population study

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Background: A new indirect bronchial provocation test measuring airway responsiveness by using inhaled mannitol was recently introduced.

Objective: The aim of this study was to examine the diagnostic properties of airway responsiveness to inhaled mannitol in the assessment of asthma in an unselected sample of young adults.

Methods: Two hundred thirty-eight young adults randomly drawn from the nationwide civil registration list were challenged with inhaled, dry-powder mannitol. A respiratory specialist, blind to the test results, classified all 238 subjects with respect to the presence of asthma. The classification was based on respiratory symptoms, spirometric results, atopy, and fraction of exhaled nitric oxide values and response to inhaled β_2 -agonists. On this basis, sensitivity, specificity, and predictive values were assessed to different cutoff values of the test. A receiver operating characteristic curve was constructed, and the accuracy of the test, defined as the area under the curve, was computed.

Results: Fifty-one (21.4%) subjects had current asthma. Of 33 subjects with airway hyperresponsiveness to mannitol, 30 had current asthma. The specificity and sensitivity were 98.4% (95% CI, 96.2% to 99.4%) and 58.8% (95% CI, 50.7% to 62.6%), respectively. The positive predictive value (PPV) and negative predictive value (NPV) were 90.9% (95% CI, 78.4% to 96.8%) and 89.8% (95% CI, 87.7% to 90.7%), respectively. The area under the receiver operating characteristic curve was 0.89 (95% CI, 0.83-0.95).

Conclusions: In an unselected sample of young adults, bronchial provocation with inhaled dry-powder mannitol had a high diagnostic specificity for the diagnosis of asthma. (*J Allergy Clin Immunol* 2009;124:928-32.)

Key words: *Bronchial hyperresponsiveness, asthma, mannitol, bronchial provocation test, epidemiology*

Airway hyperresponsiveness (AHR) is a hallmark in subjects with bronchial asthma, and the 2 seem to correlate well in many aspects of clinical disease.^{1,2} A large spectrum of protocols and

Abbreviations used

AHR: Airway hyperresponsiveness
BPT: Bronchial provocation test
FENO: Fraction of exhaled nitric oxide
ICS: Inhaled corticosteroid
PD: Provoking dose
RDR: Response dose ratio
ROC: Receiver operating characteristic

provoking agents for bronchial provocation tests (BPTs) have been developed over the past 60 years to identify AHR and to assist in the clinical diagnosis of asthma. However, measuring AHR is not, according to current guidelines, mandatory in making the diagnosis.³ This has to do with the lack of shared and test-unique operating standards, as well as the requirement of demanding and time-consuming laboratory settings.

BPTs are typically classified as either direct (eg, methacholine and histamine) or indirect (eg, mannitol, AMP, and hypertonic saline) based on their respective mechanisms of action. Whereas direct BPTs use stimuli acting directly on the smooth muscle cells of the airways, resulting in bronchoconstriction, indirect BPTs use stimuli that cause release of mediators in the airways, which in turn cause bronchoconstriction. The direct BPTs are in general sensitive, and a negative test result usually rules out asthma. However, the direct BPTs lack specificity because a positive test result can be observed in a significant number of nonasthmatic subjects, including those with chronic obstructive pulmonary disease, allergic rhinitis, and respiratory infections, as well as in asymptomatic subjects.⁴⁻⁶

Indirect BPTs, on the other hand, show high specificity in identifying current asthma compared with the direct agents. The indirect tests also correlate better with airway inflammation and severity of disease.⁷⁻⁹ Furthermore, AHR to indirect BPTs predicts short- and medium-term effects of inhaled corticosteroid (ICS) treatment in asthmatic subjects.^{1,10-12} However, although the indirect tests perform well in confirming the presence of asthma and predicting the effect of anti-inflammatory treatment, their sensitivity is lower compared with that of the direct tests.

A new BPT with inhaled mannitol was recently introduced.¹³ The test has only 1 operating standard and consists of a handheld inhaler device and 19 capsules with mannitol. No additional medical equipment, other than a spirometer, is required to carry out the test. Mannitol is categorized as an indirect test in terms of bronchoprovocation and is thought to act through an increased osmolarity in the pericilliary liquid. This results in release of bronchoconstricting mediators from inflammatory cells, which eventually causes smooth muscle contraction.¹⁴

At present, studies on mannitol have examined only selected groups of asthmatic subjects and matching control subjects. Hence the characteristics and value of mannitol in an

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TABLE I. Baseline characteristics (self-reported) in 448 subjects according to responder status

Variables	Examined population (n = 238)	Screening population (n = 210)	P value
Age (y)	18.89 (SD ± 2.95)	18.71 (SD ± 2.97)	NS
Sex			<0.05
Male	96 (40.3%)	110 (52.4%)	
Female	142 (59.7%)	100 (47.6%)	
Asthma	52 (21.8%)	32 (16.9%)	NS
Wheezing	100	69	<.05
Shortness of breath	57	36	NS
Symptoms at rest*	55	38	NS
Rhinitis	71	37	<.05
Dermatitis	68	46	NS
Familial predisposition†	128	106	NS
Smoking (>1 cigarette per daily)	52	52	NS

Values are in actual numbers (percentages) or means (SDs). All data are self-reported. NS, Not significant.

*Either wheezing, breathlessness, chest tightness, or cough.

†Parents or siblings with asthma or hay fever.

epidemiologic setting remain unknown. For this reason, the aim of this study was to examine the diagnostic properties of AHR to inhaled mannitol in the assessment of asthma in an unselected sample of young adults.

METHODS

Study design

The study is a cross-sectional population study performed at the Respiratory Research Unit, Copenhagen University Hospital Bispebjerg, Copenhagen, Denmark. An unselected sample of 1000 young adults between the ages of 14 and 24 years was randomly drawn from the civil registration list. All subjects received a validated self-administered asthma and rhinitis screening questionnaire, with 20 questions adopted from the American College of Allergy, Asthma & Immunology screening program extended with questions concerning tobacco consumption.¹⁵

At the clinic, all participants had their fraction of exhaled nitric oxide (FENO) and lung function values measured, a skin prick test and a BPT with inhaled mannitol were performed, and reversibility to inhaled β_2 -agonists was recorded. In line with international guidelines on bronchial provocation, all participants were told to withhold use of antiasthmatic drugs before the examination.¹⁶ This included use of short- and long-acting inhaled bronchodilators, leukotriene modifiers, oral bronchodilators, and ICSs.

All participants completed a symptom-based, semistructured interview at the visit based on Global Initiative for Asthma guidelines.³ Asthma-related symptoms were chest tightness, cough, wheezing, episodic breathlessness, exercise-induced dyspnea, and nocturnal symptoms. Data on rhinitis, atopic dermatitis, and familial predisposition to asthma, allergy, or both were included as well. A single investigator carried out all tests, measurements, and interviews. Further details on the study population, methods, and guidelines used in making the measurements are provided in the Methods section of this article's Online Repository at www.jacionline.org.

Mannitol bronchoprovocation test

Dry-powder mannitol (Aridol; Pharmaxis, Sydney, Australia) was administered according to the recommendations of the manufacturer, and FEV₁ was recorded in line with current guidelines.¹⁷ The FEV₁ recorded after inhalation of a 0-mg placebo capsule constituted baseline lung function. The challenge was stopped at a decrease in FEV₁ of 15% or greater from baseline values or when the maximum cumulative dose of 635 mg had been administered.

Asthma definition

A respiratory specialist performed the evaluation to ensure consistent classification of the asthma diagnosis. Interpretation of lung function tests and

other paraclinical data followed current international guidelines.^{3,18} A diagnosis of asthma was consistent with symptoms of asthma in combination with either a FENO value of greater than 30 ppb, a history of allergic rhinoconjunctivitis, dermatitis, a positive skin prick test response and familial predisposition to atopic disease, nonallergic rhinoconjunctivitis, or an FEV₁/forced vital capacity ratio of less than 75%. The examiner was blind to the results of the mannitol tests but had otherwise free access to the abovementioned clinical information presented in a database. Only subjects with asthma presenting with respiratory symptoms within the last 12 months were classified as having "current asthma"; all other participants were classified as having "no asthma."

Statistical analysis

Previsit characteristics were compared in participants and nonparticipants based on the screening questionnaires. Differences for categorical data were assessed by using χ^2 tests.

As an index for the reactivity to inhaled mannitol, the response dose ratio (RDR) was calculated. This is defined as the percentage decrease in FEV₁ after the last dose administered divided by the final cumulative dose in milligrams. Means and SDs were calculated on the basis of RDR in all subjects to determine the average response to mannitol in asthmatic and nonasthmatic subjects. A receiver operating characteristic (ROC) curve was constructed, plotting RDR against the diagnosis of asthma. The overall accuracy of the test was measured as the area under the ROC curve.¹⁹ Sensitivity, specificity, PPV, and NPV were calculated for a 10% (provoking dose [PD] 10 ≤ 635 mg) and 15% (PD15 ≤ 635 mg) decrease in FEV₁, respectively. Furthermore, a hypothetical scenario using the same diagnostic properties of the test but decreasing the prevalence of asthma to 10% was created.

RESULTS

Population characteristics

Of the 978 subjects possible to contact, 448 (45.8%) responded by returning the screening questionnaire. Among these, 238 (24.3%) volunteered to participate in the study and completed the examination.

There were no differences between participants and nonparticipants regarding the prevalence of self-reported asthma; age; familial predisposition to asthma, allergic disease, or both; and smoking habits. The ratio between male and female subjects, however, was different in the 2 groups (Table I).

Response to mannitol

Table II displays the baseline characteristics of the 238 participants. More female (59.7%) than male subjects participated in

TABLE II. Baseline characteristics in the 238 examined subjects

Variables	Male (n = 96)	Female (n = 142)	Total (n = 238)	P value
Age (y), mean (SD)	18.73 (2.95)	19.02 (2.93)	18.90 (2.94)	NS
Atopy (%)	49 (51.0)	50 (35.2)	99 (41.6)	<.05
FEV ₁ % predicted, mean (SD)	98.02 (9.54)	96.18 (11.23)	96.92 (10.60)	NS
FEV ₁ /FVC, mean (SD)	0.862 (0.06)	0.878 (0.07)	0.871 (0.06)	NS
Current asthma (%)	20 (20.8)	31 (21.8)	51 (21.4)	NS
Rhinitis	44 (46.8)	66 (46.8)	110 (46.8)	NS
FENO, mean	20.7	14.4	16.7	<.001

Values represent absolute numbers (percentages) or means (SDs). A respiratory specialist defined current asthma. NS, Not significant; FVC, forced vital capacity.

TABLE III. Characteristics of 238 random subjects provoked with inhaled dry-powder mannitol

	Current asthma	No asthma	Total
15% Reduction in FEV ₁ at ≤635 mg			
Positive test result (%)	30* (58.8)†	3* (1.6)‡	33
Negative test result (%)	21* (41.2)†	184* (98.4)‡	205
Percentage decrease in FEV ₁			
Mean (SD)	14.7 (7.3)	3.8 (4.1)	
Median	15.9	3.1	
Interquartile range	11.2	4.8	
Minimum-maximum	-1.5 to 29.7	-7.3 to 16.9	
PD15‡ (mg)			
Mean (SD)	250.2 (203.9)	430.6 (29.9)	
Median	197.3	436.5	
Interquartile range	379.4	—	
Minimum-maximum	6.2 to 602.6	398.1 to 457.1	
LogRDR			
Mean (SD)	-1.48 (0.78)	-2.77 (1.13)	
P value			<.001
10% Reduction in FEV ₁ at ≤635 mg			
Positive test result	36* (70.6)†	13* (7.0)‡	49
Negative test result	15* (29.4)†	174* (93.0)‡	189
7% Reduction in FEV ₁ at ≤635 mg			
Positive test result	42* (82.4)†	38* (20.3)‡	80
Negative test result	9* (17.6)†	149* (79.7)‡	158

PD15, Provoking dose of mannitol to cause a 15% decrease in FEV₁.

*Numbers equal individuals.

†Percentage of subjects with current asthma and nonasthmatic subjects, respectively.

‡Includes all subjects experiencing a 15% reduction in FEV₁ at a dose of 635 mg or less (asthma, n = 30; nonasthma, n = 3).

the survey. Fifty-one (21.4%) subjects had current asthma. In total, 33 subjects had AHR to mannitol, which was defined as a 15% decrease in FEV₁ at a total dose of 635 mg of mannitol or less. The overall distribution of all 238 subjects in terms of responsiveness to mannitol is presented in Table III and Figure 1.

On average, the response to mannitol in the nonasthmatic subjects was a 1.1% (95% CI, 0.8% to 1.6%) decrease in FEV₁ from baseline values, as opposed to 21.0% (95% CI, 12.7% to 34.8%) in the group of subjects with current asthma ($P < .001$).

Diagnostic properties of inhaled mannitol

Of the 51 subjects with current asthma, 30 had AHR to inhaled mannitol (ie, had a 15% reduction in FEV₁ at ≤635 mg; Table III).

This corresponds to a sensitivity of 58.8%. Three of the 187 subjects without current asthma had positive results to mannitol, corresponding to a specificity of 98.4%. The PPV and NPV were 90.9% and 89.8%, respectively. Eight subjects, all with current asthma, were currently using ICSs. Six of the 8 subjects had a PD15 of 635 mg or less.

Decreasing the cutoff limit to 10% resulted in an increased sensitivity of 70.6% and a decreased specificity of 93.0%. The sensitivity, specificity, PPV, and NPV at different cutoff values are presented in Table IV. This table also includes a hypothetical scenario, in which the prevalence of current asthma was decreased to 10%.

The overall accuracy of the mannitol test for the diagnosis of current asthma described as the area under the ROC curve (Fig 2) was 89.1% (95% CI, 83.2% to 95.0%).

DISCUSSION

Recently, a new BPT for asthma with inhalation of mannitol powder was introduced. Unlike the current tests available, the mannitol test has only 1 operating standard and protocol. The inconveniences of comparing test results from one study with those from another because of differences in protocols have remained a major weak spot in AHR research. One operating standard is therefore an absolute advantage.

It was the objective of this study to uncover the distinctive qualities of the mannitol test in an unselected, population-based setting. The study proved bronchial provocation with inhaled dry-powder mannitol to be a highly specific test with a specificity of 98.4%. Furthermore, the PPV and NPV of 91% and 90%, respectively, indicate a high validity in the diagnosis of asthma. These findings are supported by an ROC curve of 0.89, which implies that a subject with asthma has an 89% probability of being more responsive to the mannitol test than a subject without asthma. Although the test turns out to be highly specific, the sensitivity is somewhat lower. Twenty-one of 51 subjects with asthma did not respond to inhaled mannitol (ie, had a 15% reduction in FEV₁ at ≤635 mg). A subject with current asthma will consequently have a positive result to mannitol in 58.8% of cases. This is in line with the sensitivity of other indirect test for bronchial provocation, such as AMP measurement and exercise.^{6,20}

As exemplified in Table IV, the diagnostic properties of the test change toward higher sensitivity when lowering the cutoff point. Using a 15% reduction in FEV₁ as the cutoff point provides a diagnostic tool with high specificity and can be used to make a confirmed diagnosis of asthma in the case of a positive test result. On the other hand, if the objective is to rule out the

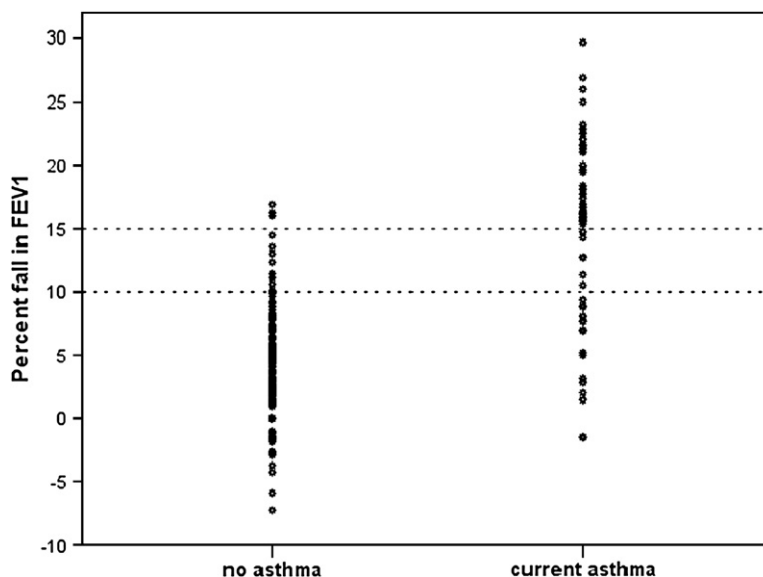


FIG 1. Reactivity to mannitol in a random population sample of asthmatic and nonasthmatic subjects. The 2 dotted lines represent cutoff points at a 10% or 15% decrease in FEV₁.

TABLE IV. Diagnostic properties of inhaled mannitol at 3 cutoff points in 238 randomly selected subjects

	Sensitivity	Specificity	PPV	NPV
PD15	58.8 (50.7-62.6)	98.4 (96.2-99.4)	90.9 (78.4-96.8)	89.8 (87.7-90.7)
PD10	70.6 (60.4-78.5)	93.0 (90.3-95.2)	73.5 (62.9-82.7)	92.1 (89.3-94.2)
PD7	82.4 (71.5-90.0)	79.7 (76.7-81.8)	52.5 (45.6-57.4)	94.3 (90.8-96.8)
Fictive scenario of 238 similar subjects but an asthma prevalence of 10%				
PD15	—	—	82.4 (62.3-93.4)	95.5 (93.9-96.3)
PD10	—	—	53.1 (40.2-62.9)	96.6 (94.6-98.1)
PD7	—	—	31.7 (25.0-35.5)	97.7 (95.5-99.1)

Numbers are in percentages (95% CIs). PD15, PD10, and PD7 represent the percentage decrease in FEV₁ (15%, 10%, or 7%, respectively) at a 635-mg cumulative dose of mannitol.

possibility of asthma, a 10% cutoff point provides a more sensitive tool, and a negative test result will help exclude the presence of asthma. However, based on these results, we are not able to conclude whether the mannitol BPT with a 10% cutoff point is equivalent or even superior in sensitivity to a standard direct BPT, such as methacholine, for cases in which asthma is to be ruled out. At present, we would therefore recommend the continued use of direct BPT for such cases.

The lack of an operating gold standard in asthma diagnostics remains the shortcoming in this research area. In this study asthma was diagnosed on the basis of asthma symptoms, para-clinical data, atopic disease, and familial predisposition. A single examiner carried out all tests and interviews, and a respiratory specialist carried out all diagnostic evaluations. This practice holds several strengths, as well as weaknesses. Most importantly, although the asthma diagnosis is consistent and can be reproduced, it might introduce a bias in terms of accuracy. Because interobserver variability cannot be assessed with only a single observer, this might be a major limitation to the study.

Baseline variability in FEV₁ 1 week before examination, as suggested for aspirin challenge,²¹ was not performed in this study. However, because baseline FEV₁ measurement and placebo challenge were performed on the day of the examination, we find no significant reason to believe variability in FEV₁ had an effect on the results of this study.

A participation rate of 25% puts the study at risk of selection bias. However, with the results of the screening questionnaire for another 25% of the invited population, a comparison of the participants and nonparticipants was possible. They did not differ in self-reported asthma, shortness of breath, symptoms at rest, familial predisposition, or smoking habits. More female than male subjects, however, chose to participate in the survey, and an overrepresentation of participants compared with nonparticipants had experienced wheezing and rhinitis. All together, there is no strong evidence that the study subjects were not representative for the population as a whole.

The prevalence of asthma in this study was higher than reported earlier in Denmark. In particular, a study from 2004 by Thomsen et al²² reported a prevalence of 11.7%, whereas the prevalence of asthma in young adults in surrounding countries, as reported by the International Study of Asthma and Allergies in Childhood study, lies between 9.7% and 19.0%.²³ In this study, however, we found no difference in the prevalence of asthma between the examined subjects and nonparticipants, indicating that the studied sample was representative of the population. Table IV displays a fictive scenario of an asthma prevalence of 10%. This shows a decreased predictive value of a positive test result, although not in reach of the values found with direct agents.^{24,25}

Finally, it needs mentioning that ICSs have an effect on reactivity to BPTs up to 6 months after withholding the drug.

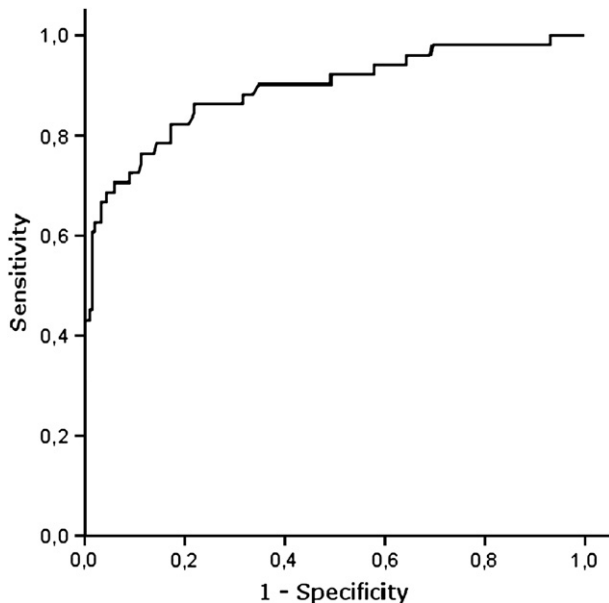


FIG 2. ROC curve. The curve visualizes reactivity to mannitol, given in RDR, against the diagnosis of current asthma.

Nevertheless, for ethical reasons, a withdrawal of ICSs was not carried out in this study for more than 12 hours. Eight subjects were receiving current ICS treatment, and 6 of them still had a positive mannitol test result. That being the case, the influence of ICSs on the results as a whole is considered minimal. The somewhat low percentage of treated asthmatic subjects in this study reflects a known excessive problem of unawareness and undertreatment of asthma in Denmark in up to 75% of all asthmatic subjects.²⁶

Clinical implications: Our results imply that bronchial provocation with inhaled mannitol for the diagnosis of asthma has a high specificity and can be used in the clinic to make a confirmed diagnosis of asthma.

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METHODS

Subjects

An unselected sample of 1000 young adults between the ages of 14 and 24 years was randomly drawn from the civil registration list. All subjects were living in Copenhagen, Denmark, at the time of the drawing. Exclusion criteria were pregnancy, emigration out of Denmark after the drawing, or respiratory infection within the past 6 weeks. All subjects received a letter with a validated screening questionnaire and an invitation to participate in the survey. The study was approved by the local ethics committee ([KF] 01 310187) and the Danish Medicines Agency (journal no. 2612-3510) and was monitored by the Good Clinical Practice Unit of Copenhagen University.

FE_{NO}

FE_{NO} was measured with a nitric oxide analyzer (NIOX; Aerocrine AB, Solna, Sweden). The procedure was carried out according to American Thoracic Society guidelines,^{E1} and a constant expiratory flow rate of 0.05 L/s was achieved by using a biofeedback monitoring system. The mean of 3 approved measurements was used in the analysis.

Skin prick tests

Skin prick tests were performed according to European Academy of Allergy and Clinical Immunology recommendations and included 10 aeroallergens (birch, grass, mugwort, horse, dog, cat, house dust mites [Der p 1 and Der f 2], and molds [*Alternaria* and *Cladosporium* species]; ALK-Abelló, Hørsholm, Denmark). Wheal size was determined 15 minutes after administration of allergens, and a wheal diameter of at least 3 mm was defined as a positive test result. Atopy was defined as at least 1 positive response to an allergen.

Lung function and the mannitol bronchoprovocation test

FEV₁ and forced vital capacity were measured on a 7L dry wedge spirometer (Vitalograph, Lenexa, Kan). All measurements of FEV₁ and forced vital

capacity were carried out according to the European Respiratory Society/American Thoracic Society recommendations.^{E2}

Interview and questionnaire

All subjects received a letter with a validated self-administered asthma and rhinitis screening questionnaire with 20 questions adopted from the American College of Allergy, Asthma & Immunology screening program and extended with questions concerning inspiratory stridor and tobacco consumption.^{E3} The subjects who completed the examination underwent a semistructured interview at the study site. The interview was built on American Thoracic Society and Global Initiative for Asthma guidelines.^{E4,E5} Any history of asthma-related symptoms, rhinitis, conjunctivitis, or atopic eczema was registered. Use of medication in relation to asthma or allergic disease was recorded along with familial predisposition to asthma, atopic diseases, or both.

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