Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma

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Abstract
Background—It is desirable to prescribe the minimal effective dose of inhaled steroids to control asthma. To ensure that inflammation is suppressed whilst using the lowest possible dose, a sensitive and specific method for assessing airway inflammation is needed.

Methods—The usefulness of exhaled nitric oxide (NO), sputum eosinophils, and methacholine airway responsiveness (PC20) for monitoring airway inflammatory changes following four weeks of treatment with an inhaled corticosteroid (budesonide via Turbohaler) were compared. Mild stable steroid naive asthmatic subjects were randomised into two double blind, placebo controlled studies. The first was a parallel group study involving three groups receiving either 100 µg/day budesonide (n = 8), 400 µg/day budesonide (n = 7), or a matched placebo (n = 6). The second was a crossover study involving 10 subjects randomised to receive 1600 µg budesonide or placebo. The groups were matched with respect to age, PC20, baseline FEV1, (% predicted), exhaled NO, and sputum eosinophilia.

Results—There were significant improvements in FEV1, following 400 µg and 1600 µg budesonide (11.3% and 6.5%, respectively, p<0.05). This was accompanied by significant reductions in eosinophil numbers in induced sputum (0.7 and 0.9 fold, p<0.05). However, levels of exhaled NO were reduced following each budesonide dose while PC20 was improved only with 1600 µg budesonide. These results suggest that exhaled NO and PC20 may not reflect the control of airway inflammation as accurately as the number of eosinophils in sputum. There were dose dependent changes in exhaled NO, sputum eosinophils, and PC20 to inhaled budesonide but a plateau response of exhaled NO was found at a dose of 400 µg daily.

Conclusion—Monitoring the number of eosinophils in induced sputum may be the most accurate guide to establish the minimum dose of inhaled steroids needed to control inflammation. This, however, requires further studies involving a larger number of patients.

Keywords: asthma; inhaled corticosteroids; airway inflammation

Inhaled glucocorticoids are the most effective therapy currently available for the treatment of chronic asthma. They are now recommended for asthmatic patients who have symptoms more than twice a week or require an inhaled β2 agonist more than once daily. High dose inhaled steroids are recommended for the treatment of more severe asthma. Once the disease is under control, the dose of inhaled steroids should be stepped down to the minimum dose that maintains control. It is desirable to prescribe the minimal effective dose of inhaled steroids to control asthma. To ensure that inflammation is suppressed whilst using the lowest possible dose, a sensitive and specific method for assessing airway inflammation is needed.

Methods—The usefulness of exhaled nitric oxide (NO), sputum eosinophils, and methacholine airway responsiveness (PC20) for monitoring airway inflammatory changes following four weeks of treatment with an inhaled corticosteroid (budesonide via Turbohaler) were compared. Mild stable steroid naive asthmatic subjects were randomised into two double blind, placebo controlled studies. The first was a parallel group study involving three groups receiving either 100 µg/day budesonide (n = 8), 400 µg/day budesonide (n = 7), or a matched placebo (n = 6). The second was a crossover study involving 10 subjects randomised to receive 1600 µg budesonide or placebo. The groups were matched with respect to age, PC20, baseline FEV1, (% predicted), exhaled NO, and sputum eosinophilia.

Results—There were significant improvements in FEV1, following 400 µg and 1600 µg budesonide (11.3% and 6.5%, respectively, p<0.05). This was accompanied by significant reductions in eosinophil numbers in induced sputum (0.7 and 0.9 fold, p<0.05). However, levels of exhaled NO were reduced following each budesonide dose while PC20 was improved only with 1600 µg budesonide. These results suggest that exhaled NO and PC20 may not reflect the control of airway inflammation as accurately as the number of eosinophils in sputum. There were dose dependent changes in exhaled NO, sputum eosinophils, and PC20 to inhaled budesonide but a plateau response of exhaled NO was found at a dose of 400 µg daily.

Conclusion—Monitoring the number of eosinophils in induced sputum may be the most accurate guide to establish the minimum dose of inhaled steroids needed to control inflammation. This, however, requires further studies involving a larger number of patients.

Keywords: asthma; inhaled corticosteroids; airway inflammation
(salbutamol) therapy on demand were recruited into the study. Stable asthma was defined as no changes in asthma symptoms and asthma medications in the previous month. Patients were required to have a prebronchodilator FEV₁ of ≥80% predicted without a history of corticosteroid treatment or an exacerbation of asthma within the previous three months. Allergic status was defined by the presence of a positive skin prick test to at least one of four common aeroallergens (grass pollen, cat dander, Dermatophagoides pteronyssinus, Aspergillus fumigatus). All patients gave a history of intermittent wheezing and chest tightness and had previously been diagnosed by a physician as having asthma. Patients had a provocative concentration of methacholine producing a 20% fall in FEV₁ (PC20) of ≤4 mg/ml. Exclusion criteria included a history of upper respiratory tract infection within six weeks of the start of the study and treatment with nasal steroids within the previous two months. The study protocol was approved by the ethics committee of the Royal Brompton Hospital.

**PROTOCOLS**

Inflammation within the airways was reduced by giving inhaled budesonide via a dry powder inhaler device (Turbohaler) at a dose of 100 µg (minimum), 400 µg (medium), and 1600 µg (maximum) to mild asthmatic subjects (four-fold different doses). This allowed us to compare the changes in exhaled NO, sputum eosinophils and PC20 in relation to the changes in lung function. At the same time we were able to determine whether inhaled budesonide inhibited these inflammatory markers in a dose dependent manner. The budesonide dose of 100 µg had to be given as one puff daily while, in those with mild to moderate stable asthma, the 400 µg dose could be given as either once daily or two divided doses. The maximum recommended dose of 1600 µg daily was given as two divided doses in order to obtain the maximum benefit with minimal side effects. Although a double parallel group study involving the three different doses of budesonide could be accomplished with added placebo, it would be complicated, requiring four Turbohaler devices for each subject. At this time we were conducting a double blind crossover study (high dose budesonide study) using budesonide Turbohaler 1600 µg daily or a matching placebo to determine the maximum benefit of budesonide on airway inflammation. This allowed us to use the data obtained before and after budesonide treatment to demonstrate its maximum effect. We then conducted another study to evaluate the effects of budesonide at lower doses (low dose budesonide study) and analysed the data from both studies together to compare the three different daily doses of budesonide. Based on the standard deviation of exhaled NO in mild asthma being 6 ppb, eight subjects were required in each budesonide treatment arm to detect the changes in exhaled NO of 9 ppb within group for an alpha specification of 0.05 and a beta specification of 0.20 (80% power).

The low dose budesonide study was a double blind randomised parallel group study. This involved three parallel groups of patients with mild asthma who received either 100 or 400 µg of budesonide Turbohaler or a matching placebo given via a Turbohaler as one puff daily. Following a one week run in period the patients were randomised to receive either placebo or budesonide Turbohaler for four weeks. Six and eight patients were required for the placebo and each budesonide treatment group, respectively. FEV₁, exhaled NO, PC20 and sputum eosinophil numbers were measured before randomisation and at the end of each treatment period.

The high dose budesonide study involved mild asthmatic subjects with the same inclusion and exclusion criteria. Patients were randomised to receive either budesonide 1600 µg daily (via Turbohaler, 400 µg/puff given as two puffs twice daily) or matching placebo for four weeks in a double blind crossover fashion. The washout period was four weeks. FEV₁, exhaled NO, PC20 and sputum eosinophil numbers were measured before and after each treatment period. Ten subjects were recruited and randomly allocated to receive either budesonide first (n = 5) or placebo first (n = 5).

In both studies subjects recorded morning and evening peak expiratory flow rate (PEF, best of three), symptom scores, and the amount of rescue inhaled β₂ agonist (puffs per day) throughout the study period. Symptom scores were measured as asthma during the day, asthma during the night, and early morning tightness, ranging from 0–3 for each item (0 = none, 1 = mild, 2 = moderate, 3 = severe).

**LUNG FUNCTION**

FEV₁, and FVC were measured with a dry spirometer (Vitalograph, Buckingham, UK). The best value of the three manoeuvres was expressed as a percentage of the predicted value. Morning and evening peak flow were measured using a mini-Wright peak flow meter (Clement Clarke International Ltd, Harlow, UK).

**AIRWAY RESPONSIVENESS**

Airway responsiveness was measured by methacholine challenge with doubling concentrations of methacholine (0.06–32 mg/ml) delivered by dosimeter (Mefar, Bovezzo, Italy) with an output of 10 µl per inhalation. The aerosols were inhaled at tidal breathing while wearing a nose clip. A total of five inhalations of each concentration was administered (inhalation time one second, breath holding time six seconds). FEV₁ was measured two minutes after the last inhalation until there was a fall in FEV₁ of ≥20% compared with the control inhalation (0.9% saline solution) or until the maximal concentration was inhaled. The PC₂₀ was calculated by interpolation of the logarithmic dose response curve.

**MEASUREMENT OF EXHALED NO**

End exhaled NO was measured by a chemiluminescence analyser (Model LR2000, Logan...
Sputum induction and processing

Sputum was collected using the method previously described by Keatings et al. Subjects were instructed to wash their mouths thoroughly with water prior to induction. They then inhaled 3.5% saline at room temperature, nebulised via an ultrasonic nebuliser (DeVilbiss 90, DeVilbiss, Heston, UK) at maximum output for 15 minutes. Subjects were encouraged to cough deeply at five and three minute intervals thereafter. Sputum was collected into a polypropylene pot and saliva was discarded into a bowl. Following sputum induction the spirometric measurements were repeated. If FEV₁ had fallen, the subject was required to wait until it had returned to the baseline value. Sputum samples were kept at 4°C for not more than two hours before further processing.

The volume of sample was recorded and the sputum was diluted with 2 ml of Hank's balanced salt solution (HBSS) containing 1% dithiothreitol (DTT; Sigma Chemicals, Poole, UK), periodically aspirated through a small bore pipette and vortexed. When homogeneously sampled, subjects were further diluted with HBSS, vortexed briefly, and left at room temperature for five minutes. They were then spun at 300g for 10 minutes and the cell pellet was resuspended with HBSS. Total cell counts were done on a haemacytometer using Kimura stain and slides were made with a cytopsin (Shandon, Runcorn, UK) and stained with May-Grunwald-Giemsa stain for differential cell counts which were performed by an observer blind to the clinical characteristics of the subjects. At least 500 inflammatory cells were counted in each subject. The reproducibility of differential cell counts in our laboratory involving 20 pairs of samples collected from stable asthmatic subjects during a two week period showed intra-class correlation coefficients of 0.75 for eosinophils, 0.78 for neutrophils, 0.76 for macrophages, and 0.56 for lymphocytes.

### Table 1  Baseline lung function and markers of airway inflammation in the four groups of asthmatic patients studied

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>100 µg</th>
<th>400 µg</th>
<th>1600 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Sex</td>
<td>6M</td>
<td>8M</td>
<td>7M</td>
<td>8F/2M</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 (2.8)</td>
<td>31 (1.2)</td>
<td>29 (2.4)</td>
<td>29 (1.2)</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>97.2 (4.0)</td>
<td>92.3 (3.1)</td>
<td>91.5 (4.2)</td>
<td>96.2 (3.1)</td>
</tr>
<tr>
<td>Morning PEF (l/min)</td>
<td>552 (32)</td>
<td>512 (20)</td>
<td>501 (19)</td>
<td>461 (33)</td>
</tr>
<tr>
<td>PEF variability (%)</td>
<td>10.7 (1.8)</td>
<td>11.9 (2.7)</td>
<td>16.7 (2.8)</td>
<td>9.3 (1.3)</td>
</tr>
<tr>
<td>Symptom scores</td>
<td>1.0 (0.4)</td>
<td>1.0 (0.3)</td>
<td>1.0 (0.4)</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>Rescue inhaler (puff/day)</td>
<td>0.9 (0.4)</td>
<td>0.8 (0.3)</td>
<td>1.0 (0.3)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>PC₉₀ (ppm)</td>
<td>0.46 (1.61)</td>
<td>0.47 (1.41)</td>
<td>0.51 (1.20)</td>
<td>0.57 (1.42)</td>
</tr>
<tr>
<td>Exhaled NO (ppb)</td>
<td>27.2 (3.5)</td>
<td>28.8 (2.4)</td>
<td>31.8 (4.1)</td>
<td>40.9 (7.2)</td>
</tr>
<tr>
<td>Sputum eosinophils (%)</td>
<td>1.9 (8.2)</td>
<td>4.9 (8.0)</td>
<td>3.5 (3.2)</td>
<td>2.2 (8.7)</td>
</tr>
</tbody>
</table>

Mean (SE) values are shown except *median value (interquartile range).*
### Table 2  Effects of inhaled budesonide treatment on markers of airway inflammation and lung function

<table>
<thead>
<tr>
<th></th>
<th>Low dose budesonide study</th>
<th>High dose budesonide study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>100 µg budesonide</td>
</tr>
<tr>
<td>FEV1 (l/min)</td>
<td>−0.2 (0.1)</td>
<td>0.0 (0.1)</td>
</tr>
<tr>
<td>% change</td>
<td>−5.8 (4.1)</td>
<td>0.2 (1.9)</td>
</tr>
<tr>
<td>morning PEF (l/min)</td>
<td>−17 (10)</td>
<td>20 (5)</td>
</tr>
<tr>
<td>% change</td>
<td>−2.9 (1.7)</td>
<td>4.1 (1.2)</td>
</tr>
<tr>
<td>Symptom scores</td>
<td>0.1 (0.5)</td>
<td>−0.4 (0.2)</td>
</tr>
<tr>
<td>Rescue inhaler (puff/day)</td>
<td>0.6 (0.2)</td>
<td>−0.5 (0.3)</td>
</tr>
<tr>
<td>PC20 (mg/ml)</td>
<td>−0.69 (2.00)</td>
<td>1.01 (1.57)</td>
</tr>
<tr>
<td>Fold change in log PC20</td>
<td>−2.4 (6.4)</td>
<td>−22.5 (6.3)</td>
</tr>
<tr>
<td>Sputum eosinophil number (%)‡</td>
<td>0.1 (0.1)</td>
<td>−0.2 (0.1)</td>
</tr>
<tr>
<td>Fold change§</td>
<td>3.7 (5.1)</td>
<td>−0.6 (3.8)</td>
</tr>
</tbody>
</table>

Abbreviations as in table 1.
*Significant difference between three treatment groups by one way ANOVA or Kruskal-Wallis test.
†Percentage or fold changes from baselines were used for comparisons.
‡Differences between the values measured at the end of first treatment period (either budesonide or placebo) subtracted by the same values measured at the end of the second treatment period (either placebo or budesonide); each value indicates the average or median change from baseline in five subjects who received budesonide first followed by placebo (Bud–Pla) and vice versa (Pla–Bud).
**Treatment effects obtained by comparing the differences between (Bud–Pla) and (Pla–Bud).
¶Geometric CI.
§Summarises the changes from baseline in 10 subjects.

**Low dose budesonide study**

Exhaled NO levels were significantly reduced following both 100 µg budesonide (from 28.8 to 20.6 ppb) and 400 µg budesonide (from 31.8 to 15.8 ppb) but remained unchanged following placebo treatment (from 27.2 to 28.7 ppb). Within each treatment comparison there were significant reductions following treatment with both 100 µg (p < 0.05, 95% CI 1.7 to 14.5) and 400 µg (p < 0.01, 95% CI 6.9 to 31.4) budesonide. The mean fold changes from baseline were −0.2, −0.6, and 0.1 following 100 µg, 400 µg budesonide and placebo, respectively. Between treatment comparison showed a significant difference only between the placebo and 400 µg budesonide groups (p<0.01, 95% CI −1.1 to −0.3, table 2, fig 1A, left panel).

There was a reduction in the median number of sputum eosinophils following both 100 µg budesonide (from 4.9% to 1.5%) and 400 µg budesonide (from 3.5% to 1.0%) but the eosinophil number was increased following placebo treatment (from 1.9% to 5.2%). Within each treatment comparison there was only a significant reduction after 400 µg budesonide (p<0.05, 95% CI 0.3 to 3.8). The median fold changes from baseline were −0.6, −0.7, and 3.7 after 100 µg budesonide, 400 µg budesonide and placebo, respectively. Between treatment comparisons demonstrated a significant difference between the placebo and 400 µg budesonide groups (p<0.05, 95% CI 0.2 to 5.8, table 2, fig 1B, left panel).

FEV1 was increased following treatment with both 100 µg budesonide (from 3.8 to 3.9 l) and 400 µg budesonide (from 4.1 to 4.6 l) but decreased in the placebo treated group (from 4.0 to 3.7 l). The mean percentage increases in FEV1 were 1.2%, 11.3%, and −5.8% following 100 µg, 400 µg budesonide and placebo, respectively. Within each treatment comparison there was a significant improvement only after 400 µg budesonide (p<0.05, 95% CI −0.9 to −0.1). Comparison between treatments showed a significant difference between placebo and 400 µg budesonide treatment only (p<0.01, 95% CI −20.9 to −5.3, table 2, fig 2A, left panel). Similarly, morning PEF was significantly increased following treatment with 400 µg budesonide compared...
There were reductions in sputum eosinophil numbers from 2.2 (8.7)% to 0.2 (1.5)% following the four week treatment with 1600 µg budesonide. The median change (fold) in sputum eosinophil number following 100, 400, and 1600 µg budesonide were –0.6, –0.7, and –0.9, respectively. Analysis for a trend across the groups showed a significant trend towards more reduction in sputum eosinophils with increasing doses of budesonide (p<0.05). This suggested a greater reduction in sputum eosinophil numbers with increasing dose of inhaled budesonide (fig 1B).

With the treatment period of four weeks the increases in \( PC_{20} \) (geometric mean, mg/ml) from baseline following treatment with 100, 400, and 1600 µg budesonide were 1.01 (1.57), 1.31 (1.51), and 6.57 (1.50), respectively. Analysis for a trend across the groups demonstrated a greater improvement in \( PC_{20} \) with increasing doses of budesonide (p<0.01; fig 1C).

**Discussion**

In this composite study we have shown that monitoring exhaled NO and sputum eosinophils may be useful in the assessment of airway inflammatory changes following inhaled corticosteroid treatment. There were dose dependent changes in sputum eosinophils and \( PC_{20} \) to inhaled budesonide, with the maximum reduction at the highest dose. Exhaled NO levels were also decreased in a dose dependent manner but the maximum suppression was reached with the medium dose of budesonide.

We have shown that the use of budesonide in a daily dose of 100 µg led to a significant reduction in exhaled NO levels compared with baseline, yet there was no significant change in lung function and other non-invasive markers of inflammation such as sputum eosinophilia and \( PC_{20} \). Although it is possible that a significant reduction in sputum eosinophil numbers would have been statistically significant if a larger number of subjects had been included, this suggests that NO may be more sensitive to low doses of inhaled steroids. A reduction in exhaled NO following treatment with inhaled corticosteroids may not therefore necessarily reflect a control of airway inflammation and needs to be confirmed by more direct measurements such as sputum eosinophil number. Our data have shown a dose dependent effect on exhaled NO, as budesonide 400 µg was more effective in reducing NO than budesonide 100 µg. However, there was no further reduction with the dose of 1600 µg, possibly due to a plateau response of exhaled NO to higher doses of inhaled steroids. This plateau in response of exhaled NO in the face of further changes in other inflammatory markers such as sputum eosinophils and \( PC_{20} \) may limit the clinical usefulness of exhaled NO as an accurate marker for monitoring asthma control as it may be too sensitive to inhaled corticosteroids. However, it needs to be emphasised that only
mild steroid naive asthmatic subjects were studied.

Sputum induction has been advocated as a non-invasive alternative for measuring airway inflammation with greater advantage in terms of reproducibility and simplicity. The number of eosinophils in sputum has been found to correlate with asthma severity. Eosinophil numbers are increased in both mild and severe exacerbations of asthma, but they are decreased with corticosteroid treatment in association with an improvement in lung function. This affirms the potential value of sputum eosinophils as an objective marker for assessing the control of asthma. Our study supports this conclusion, as a significant reduction in sputum eosinophils was found only in association with a significant improvement in FEV1. In contrast, there was an increase in sputum eosinophils in association with poor asthma control in placebo treated patients. This suggests that there is persistent variable eosinophilic inflammation within the airways of asthmatic subjects not treated with inhaled steroids. If airway inflammation is not monitored, this unrecognised inflammation might lead to irreversible airway damage over time. The inhibitory effect of corticosteroids on sputum eosinophils could be due to an inhibitory effect of steroids to the permissive action of cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin-5 (IL-5) on eosinophil survival, a reduction in circulating eosinophil numbers, and a reduction in the concentration of IL-5 in sputum and blood.

There is clinical evidence to suggest that inhaled steroids improve asthma control in a dose related manner and high dose inhaled steroids are recommended for more severe asthma. However, no clear dose response effect of inhaled steroids on airway inflammation has yet been demonstrated. This may be due to the heterogeneity of patients recruited, the varying degree of airway drug deposition, or lack of available sensitive methods for measuring airway inflammation. Our mild asthmatic subjects had the same clinical severity by conventional markers of asthma severity such as lung function, peak flow variation, and asthma symptom scores. Moreover, they had the same basal levels of airway inflammation reflected by sputum eosinophil numbers, PC20 and exhaled NO levels. In this study we have shown a significant trend towards greater reduction in sputum eosinophils with higher dose budesonide, suggesting a dose dependent effect of inhaled steroids on eosinophilic airway inflammation. It remains to be established whether in mild asthma the differing dose schedules may partly account for a greater effect of the higher doses of budesonide. The studies in patients with mild to moderately severe asthma, however, indicate that budesonide Turbonhaler 400 µg and 800 µg given once daily provide improvements in lung function to the same level as the same total daily dose given twice daily. It is also possible that budesonide in a dose of 100 µg daily may lead to a significant reduction in sputum eosinophils with a larger number of patients treated for a longer period, as the anti-inflammatory effect of inhaled steroids is also time dependent.

Airway inflammation contributes to airway hyperresponsiveness. By suppressing inflammation within the airways, corticosteroids improve asthma control and airway hyperresponsiveness. The improvement in lung function usually precedes and reaches a plateau before the reduction in airway responsiveness. The reduction in responsiveness takes place over several weeks and may not be maximum for three months or, in some patients, even longer. The response of PC20 to inhaled steroids is variable between patients, but the average increase is in the order of one or two doubling dilutions. We have shown a dose dependent effect of PC20 to inhaled corticosteroids which is in agreement with previous studies. A marked increase in methacholine PC20 with budesonide 1600 µg was shown but there was no significant change with either 100 µg or 400 µg budesonide. This implies that the mechanisms underlying airway hyperresponsiveness may be less sensitive to steroid treatment. A greater improvement in PC20 with high dose inhaled steroids has been reported previously. PC20 may therefore be a less sensitive marker for monitoring the anti-inflammatory effects of corticosteroids.

Airway inflammation may not be optimally controlled with current asthma treatment guidelines. It remains unclear whether a long term complication such as irreversible airway damage can be reduced or prevented if treatment strategy is aimed at suppressing airway inflammation maximally, as guided by sputum eosinophil number or PC20. As PC20 may correlate with features of airway fibrosis, it may be desirable if asthma treatment is directed to normalise PC20. Our findings, however, indicate that high doses of inhaled steroids may be required to reduce the PC20, thus increasing the risk of systemic side effects. It may be more rational to normalise sputum eosinophil numbers at a lower steroid dose. This may also improve PC20 with chronic treatment. However, this remains to be established in further long term studies.

We conclude that exhaled NO is the most sensitive inflammatory marker for assessing the anti-inflammatory effects of inhaled steroids in steroid naive asthmatic subjects. However, the reduction in exhaled NO following treatment with inhaled steroids may not ensure that airway inflammation is optimally suppressed. This requires an additional assessment of a more direct marker of airway inflammation such as eosinophil number in induced sputum. The clinical usefulness of these markers in the management of asthma remains to be determined.

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et al.


