Are Brain Volumes based on Magnetic Resonance Imaging Mediators of the Associations of Cumulative Lead Dose with Cognitive Function?

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The authors used cross-sectional data (2001–2003) to consider the pathway through which past occupational lead exposure impacts cognitive function. They were motivated by studies linking cumulative lead dose with brain volumes, volumes with cognitive function, and lead dose with cognitive function. It was hypothesized that the brain regions associated with lead mediate a portion of the relation between lead dose and cognitive function. Data were derived from an ongoing US study of 513 former organolead manufacturing workers. Magnetic resonance imaging was used to perform a novel analysis to investigate mediation. Volumes associated with cognitive function and lead dose were derived by using registered images and were used in a subsequent mediation analysis. Cumulative lead dose was associated with adverse function in the visuo-construction, executive function, and eye-hand coordination domains. Regarding these domains, there was strong evidence of volumetric mediation of lead’s effect on cognition in the visuo-construction domain and a moderate amount for executive function and eye-hand coordination. A second path-analysis-based approach was also used. To address the possibility that chance associations explained these findings, a permuted analysis was conducted, the results of which supported the mediation inferences. The approach to evaluating volumetric mediation may have general applicability in epidemiologic neuroimaging settings.

epidemiologic factors; epidemiologic methods; lead; magnetic resonance imaging; neurobehavioral manifestations; spectrometry, X-ray emission

Abbreviations: MRI, magnetic resonance imaging; RAVENS, regional analysis of volumes in normalized space.

We previously reported that past occupational exposure to organic and inorganic lead was associated with decreased cognitive function (1) and with a decrease in the volume of brain structures as measured with magnetic resonance imaging (MRI) (2). Specifically, past cumulative absorption of lead was associated with pronounced longitudinal declines in verbal memory and learning, visual memory, and executive function and with a decrease in the volume of total brain,

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parietal white and gray matter, temporal white matter, and two paralimbic system structures (cingulate gyrus, insula), among others. These findings raise questions about the extent to which declines in cognitive function measures associated with tibia lead are mediated through brain volumes.

Our previous work indicated that lead dose was associated with persistent and possibly progressive changes in the brain, in terms of both cognition and brain structure, long after lead levels had declined (1–9). On the basis of these findings, we hypothesized that the brain regions associated with lead should mediate a portion of the association between lead and measures of cognitive function, recognizing that MRI measures only volume differences, not more subtle neurobiologic changes to brain structure. More specifically, we hypothesized that increases in lead dose are associated with measurable localized decreases in brain volume that, in turn, mediate declines in cognitive function. Providing evidence that the effect of lead on cognitive function may be mediated through losses of brain volume that are at least persistent over time should allow hypotheses to be generated about the mechanism of lead’s influence on adult cognitive function and motivate basic research into these mechanisms. The findings may also be generalizable to understanding other central nervous system insults, volume loss, and cognitive dysfunction.

We assumed that cognitive function is mediated through brain structure and neurobiology, whether or not we are able to observe all aspects of mediation. It was hypothesized that a component of the cross-sectional negative association of brain volume with lead dose mediates a decline in cognitive function. In this paper, we argue that this decline can be assessed, indirectly, by considering cognitive function at cross-section. That is, because our evidence indicated that the effect of lead is at least persistent, and possibly progressive, the variation in cognitive function observed at cross-section incorporates historical changes partially attributable to past lead exposure; the same assumption holds for MRI measures of volume. Given this framework, we investigated the hypothesis of mediation by using cross-sectional measures of historical lead dose, brain volumes, and cognitive function. In this paper, we present a novel exploratory analysis that utilizes the full information from the MRIs while not being constrained by arbitrary anatomic definitions.

MATERIALS AND METHODS

Study population, design, and data

Overview. The data for this paper were derived from an ongoing prospective study of past occupational lead exposure and its associated health effects. Subjects were recruited from a chemical manufacturing plant that previously produced gasoline additives (7). Subject recruitment occurred in two phases between 1994 and 2003. Neurobehavioral function was tested annually. Current tibia lead was measured from all participants. The study was reviewed and approved by the Johns Hopkins Bloomberg School of Public Health Committee on Human Research, and written informed consent was obtained from all participants.

Subject recruitment. Individuals recruited for this study were former workers in the organolead area of a chemical plant involved in the manufacture of tetraethyl lead from 1923 to 1991 and tetramethyl lead from 1960 to 1983, but they were not occupationally exposed to lead at study enrollment. All study participants were previously employed in the facility on or after January 1, 1950; were male; and were between the ages of 40 and 70 years in 1995. In phase 1, a total of 703 former lead workers were enrolled and completed one to four visits. In phase 2, another 276 former lead workers were enrolled and completed one or two visits. During phase 2, MRIs were completed for 589 of the 979 former lead workers. Tibia lead was measured in 532 of the 589 individuals who underwent MRI. Analysis was limited to the 513 subjects for whom there were no errors in the processing of their images and key covariate data were available. One subject was eliminated from the study because of a potential neurologic disorder. Cognitive function did not differ by MRI acquisition status, and the relations of tibia lead with neurobehavioral test scores did not differ among those with and without MRIs (2).

Data collection. Data collection, cognitive assessment, and MRI acquisition protocols for the phase 1 and phase 2 studies have been previously described (1, 2). All subjects were scanned by using the same General Electric 1.5T Signa Model (General Electric Company, Fairfield, Connecticut). Neurobehavioral test scores were aggregated into six cognitive domains: visuo-construction, verbal memory and learning, visual memory, executive function, eye-hand coordination, and processing speed, as previously described (10), according to neuropsychologic theory and empirical evaluation of inter-test correlations and variation. Before the aggregated domain measures were created, the individual test scores were normalized within the sample. This normalization included controls not considered in the current analysis. Current tibia lead was used to derive peak tibia lead levels, the estimated level at the end of occupational lead exposure, by using previously published methods (3, 11). Peak tibia lead was found to be the lead dose measure most associated with cognitive test scores (3), decline in cognitive function over time (1), brain volumes, and white matter lesions (2).

Image processing. Images were preprocessed by using previously published methods (2, 12) and were subsequently segmented into gray matter, white matter, and cerebrospinal fluid (13). Regional analysis of volumes in normalized space (RAVENS) was used to warp brain images into a standard template space while retaining volumetric information (14). That is, RAVENS images contained volumes in a standardized space (15). Hence, the shape of each subject’s image (ideally) was identical to the template brain image; however, the image intensities contained localized volume information to be compared across subjects. These images are
typically analyzed one voxel (three-dimensional pixel) at a time, across subjects.

The principal benefit of this approach is the ability to assess associations of predictor variables in highly localized volumetric analyses without relying on anatomically constrained regions of interest (16, 17). In contrast, analysis of anatomy- or function-based regions of interest requires assumptions regarding the defining qualities of the region as

**FIGURE 1.** Coronal (top left), sagittal (top right), and transverse (bottom left) projections of thresholded p values (<0.001) for gray-matter and white-matter associations of peak tibia lead and cognitive domain scores with volume, Delaware and New Jersey, 2001–2003. ROI, region of interest; EHC, eye-hand coordination.
well as the degree of anatomic resolution to be considered. Voxel-by-voxel statistical analysis of the RAVENS images enables an unbiased evaluation of brain shape and volume and post hoc determination of regions of interest not known a priori. However, voxel-based approaches come at the cost of multiplicity and other concerns arising from the massive complex data being considered at such a fine level of detail.

Table 1. Summary statistics for the volumes∗ associated with peak tibia lead and cognitive domain scores for gray matter and white matter for both regular and a representative permutation analysis of 513 former organolead workers, Delaware and New Jersey, 2001–2003

<table>
<thead>
<tr>
<th>Volume (cm³, mean (SD))</th>
<th>Gray matter</th>
<th>White matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regular analysis</td>
<td>Permuted analysis</td>
</tr>
<tr>
<td>Peak tibia lead association volume</td>
<td>9.24 (1.14)</td>
<td>0.01 (0.00)</td>
</tr>
<tr>
<td>Cognitive domain association volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visuo-construction</td>
<td>109.36 (12.17)</td>
<td>1.21 (0.15)</td>
</tr>
<tr>
<td>Verbal memory and learning</td>
<td>0.01 (0.00)</td>
<td>0.08 (0.02)</td>
</tr>
<tr>
<td>Visual memory</td>
<td>58.92 (7.65)</td>
<td>0.19 (0.03)</td>
</tr>
<tr>
<td>Executive function</td>
<td>86.93 (9.49)</td>
<td>0.66 (0.13)</td>
</tr>
<tr>
<td>Eye-hand coordination</td>
<td>24.68 (3.08)</td>
<td>5.66 (0.65)</td>
</tr>
<tr>
<td>Processing speed</td>
<td>32.74 (3.86)</td>
<td>22.96 (3.89)</td>
</tr>
</tbody>
</table>

* The volumes were derived by using a p-value threshold of 0.001 from statistical maps obtained by regressing voxel volumes from images of regional analysis of volumes in normalized space on either peak tibia lead or cognitive domain scores.
† SD, standard deviation.

Statistical analysis

Overview and justification. Our strategy for analysis was to develop novel methodology for recasting the problem in the terms of traditional mediation analysis by condensing the unmanageable multivariate images with targeted, simpler, composite summaries. As a consequence, our approach was largely exploratory, providing only indications of the existence and extent of mediation. Below is a brief overview of the analysis methods, which is followed by detailed discussions of creation and analysis of the summaries.

The analysis procedure followed three steps. First, inter-subject lead- and domain-derived association areas were identified by regressing the RAVENS voxel intensities on peak tibia lead and cognitive domain scores, respectively, and thresholding the results. Second, the subject-specific volumes contained within the association areas were calculated to obtain lead- and domain-defined subject-specific association volumes. Finally, these composite summaries were used in a mediation analysis.

Mediation is often assessed by using a general linear model in which the association between a specific predictor variable and outcome variable is evaluated with and without the potential mediator in the model (18). A large change in the magnitude of the effect was taken as evidence of mediation. Alternatively, path analysis has been used, in which linear structural associations are assumed between the outcome of interest, the mediator, and the remaining variables (19, 20). Both techniques were used in our study.

Association volumes. The construction of the association volumes deserves special attention. Conceptually, the following volumetric regions are of primary interest: the volume most associated with peak tibia lead and the volumes most associated with cognitive domain scores. The lead association volume was derived after a voxel-by-voxel regression analysis of the RAVENS maps with voxel-level volume as the outcome and peak tibia lead as the predictor. Separate regression analyses were performed with white-and gray-matter volumes as the dependent variables and peak tibia lead as the predictor, adjusting for age. This minimal adjustment including only age was used because other potential confounding variables were controlled for in the subsequent evaluation of mediation. Because of imperfect registration and noise, the RAVENS maps were smoothed by using a 10-mm, full width at half maximum Gaussian smoother, and the models were fit at only those voxels containing values for at least 400 subjects.

Voxels having t statistics below either the 0.001 or the 0.00002 normal quantiles were used to create the areas of interest. These thresholds were chosen to be consistent with our prior work where moderate and stricter thresholds were derived from false discovery rates (21). Note that interest lay in negative associations. The gray- and white-matter volumes for each subject within the areas of interest were calculated by summing the relevant voxels of each subject’s RAVENS image. The volumes, expressed in cubic millimeters, represented the gray- or white-matter volume for that subject most strongly associated with peak tibia lead across subjects.

The cognitive domain association volumes were derived in the same manner, with the following two exceptions. First, the cognitive domain scores were treated as the outcomes and the voxel-level volumes as the predictors in the voxel-wise regression models. Second, positive associations were considered when creating the areas of interest.

Evaluation of mediation. The association volumes for gray and white matter and for lead and cognitive domain
scores were included and excluded (separately) in models relating lead exposure to the cognitive domain scores, adjusting for covariates (age, visit number, education (less than high school, high school graduate, some college, college graduate), and cognitive function tester). The impact of inclusion of the association volumes on the magnitude and significance of the regression coefficient for peak tibia lead was then evaluated.

To investigate potential proportional changes in volume, we also considered models with total gray matter and total white matter included as covariates. Finally, we also considered models with height, weight, and body mass index as covariates to control for variation in intrinsic brain size.

To complement the informal regression analysis, a second, more formal analysis derived the proportion of the total effect of lead that was an indirect effect through the use of the path analysis models. Further information on path analysis approaches to mediation can be found elsewhere (20).

\[ CDS = B_0 + B_1 \text{PTL} + B_2 \text{ROI} + B_3 \text{Age} + B_4 \text{Visit} + B_5 \text{Education} + B_7 \text{Tech} + \text{Error} \]  

\[ AV = \gamma_0 + \gamma_1 \text{PTL} + \gamma_2 \text{Height} + \gamma_3 \text{Age} + \gamma_4 \text{Education} + \text{Error}, \]  

where CDS is cognitive domain score, PTL is peak tibia lead, ROI is region of interest, Education is years of education, Tech is cognitive tester, and AV is association volume (refer to Schwartz et al. (1) and Stewart et al. (3)). The direct effect is defined as \( B_1 \), the indirect effect as \( \gamma_1 B_2 \), the total effect as \( B_1 + \gamma_1 B_2 \), and the proportion of the total effect that is indirect as \( \gamma_1 B_2/(B_1 + \gamma_1 B_2) \). This and the subsequent regression models and path models were estimated by assuming mutual independence and normality of the errors using the language R (22). The voxel-wise regression models were verified, and the Gaussian smoother was applied by using the SPM package for Matlab, version 6.5 (MathWorks, Natick, Massachusetts).

One limitation of our method arose because the same data were used to create the association volumes and to evaluate mediation, with the possibility that chance associations were responsible for the results. To address this concern, we recalculated the association masks, with the labels linking subjects to their images permuted. Thus, the subsequent mediation analysis was based entirely on spurious correlations of peak tibia lead or cognitive domain scores with...
voxel volumes. One hundred such permutation replications were performed.

RESULTS

Association volumes

The association volumes on coronal, sagittal, and transverse projection images are graphically displayed in figure 1. The association volume with peak tibia lead was larger for white matter than for gray matter, and the lead association volumes were relatively small compared with the domain volumes, except for the verbal memory and learning domain (table 1). For the domain association volumes, the visuo-construction domain was the largest gray matter region, whereas processing speed was the largest in white matter. Performance in the verbal memory and learning region was not associated with gray- or white-matter volumes. The association volumes for both lead and cognitive domain scores were very small in the representative permuted analysis, except for the volumes regarding gray matter processing speed, which were still notably smaller than the actual volumes.

Description of study subjects

The 513 subjects in the analysis were all male and had an average age of 60.4 years, 8.6 years of occupational exposure to lead, 18.0 years since last exposure, and peak tibia lead level of 24 μg of lead per gram of bone mineral (table 2). The average total brain volume was 1,150.3 cm³: 588.4 cm³ of gray matter and 561.9 cm³ of white matter. On average, the date of MRI was quite close to that of the cognitive function testing (within 21.5 days), although, for a few subjects, the two dates were approximately 6 months apart. The majority of study subjects were current alcohol drinkers (69 percent), did not carry the apolipoprotein E ε4 allele (73 percent), had at least some high school education (59 percent), were White (92 percent), and were previous tobacco users (51 percent) (table 3).

Correlations among important predictor variables are summarized in table 4, including the association volumes (p-value threshold = 0.001), age, peak tibia lead level, and cognitive domain scores. Peak tibia lead and age were negatively correlated with all of the cognitive domain scores. Peak tibia lead was negatively correlated with the domain association volumes, despite not being involved in their derivation. Age was negatively correlated with all association volumes, likely because of a diffuse whole-brain volumetric loss due to normal aging. The association volumes were strongly correlated to each other, likely because of the high degree of variation in individual brain size. Of note, the verbal memory and learning domain showed lower associations overall. In addition, the cognitive domain scores were only modestly correlated with one another.

Evaluation of mediation

Evaluation of mediation is principally of interest when the total effect is significant. In regression models adjusting for covariates, peak tibia lead was associated with worse
Some evidence of mediation was present in all three of the domains, with a significant total effect of peak tibia lead. However, the visuo-construction domain showed the most striking evidence of mediation because, for both gray and white matter, the association of tibia lead with cognitive function decreased in magnitude and statistical significance after inclusion of either the lead or the domain association volume.

In the path analysis, the resulting proportion of the total effect of peak tibia lead that was indirect was 0.31, 0.23, and 0.18, and 0.18 corresponding to the association volume ordering from top to bottom in table 6, respectively. Each had a bootstrap estimated confidence interval of greater than zero.

Relatively weaker evidence of mediation was demonstrated in the executive function and eye-hand coordination domains. For executive function, the strongest suggestion of mediation was in the lead association volume for gray matter, with the peak tibia lead association becoming nonsignificant after its inclusion and an estimated 24 percent of the total effect being direct. After inclusion of the remaining association volumes, evidence of mediation was weaker, and the direct effect of peak tibia lead remained significant. Mediation through gray matter volumes was stronger than through white matter volumes, and through lead volumes was greater than through domain volumes, in both the regression and path analysis approaches. This finding is especially interesting given the much smaller average sizes of the lead volumes compared with the domain volumes.

Eye-hand coordination showed moderate evidence of mediation. The lead effect became barely nonsignificant after inclusion of the association volumes and with proportions of the total effect of peak tibia lead that were indirect of 0.19, 0.18, and 0.18 corresponding to the association volume ordering from top to bottom in table 6, respectively. Each had a bootstrap estimated confidence interval of greater than zero.
DISCUSSION

Of the three domains in which there was an association of peak tibia lead with test scores, the results suggest strong evidence of mediation in the visuo-construction domain and modest evidence of mediation in the eye-hand coordination and executive function domains. The remaining cognitive domains showed no association with peak tibia lead; hence, the mediation question was not of interest.

The approach to identification of the lead- and cognitive-domain-association volumes implemented in this study offers a way to address the question of mediation complementary to using anatomically or functionally defined regions of interest. Moreover, using flexible voxel-wise regression models to define association volumes eliminates the decision about how finely to dissect the anatomically based volumes, which can vary in size from total brain volume to the volume of small limbic substructures. In addition, our approach explicitly targets potential mediation volumes most likely affected by lead exposure and most strongly associated with cognitive domain function. In contrast, anatomically based volumes aggregate relevant and nonrelevant volumes within a specific anatomic structure that may not be related to either lead dose or cognitive function. Another notable benefit of the proposed analysis is the high degree of data reduction for the subsequent evaluation of mediation.

A principal concern regarding construction of the association volumes is that chance associations could suggest mediation not actually present. However, the permutation analysis demonstrated that the evidenced mediation is likely more substantive. An additional concern is that the methods relied heavily on smoothing the RAVENS images prior to analysis, both to compensate for imperfect registration and to counter the fact that localization to the voxel level was not warranted. Hence, the choice of the smoothing bandwidth could have impacted results.

Furthermore, the methods presented relied on assumptions such as linearity of the structural equations, Gaussian errors, and absence of other mediators and interactions. For example, biochemical or other biologic changes, such as changes in neurotransmitter release or uptake, might be an important way in which the cognitive effects of lead are evidenced in the brain. In addition, measurement error, in imaging, lead measurement, and cognitive function assessment, may play an important role. Specifically, such errors may contribute to the overall null results for verbal memory and learning. Also note that modern causal reasoning could be used to refine our statements of mediation (23, 24). For example, one must consider the possibility that our regression-adjustment approaches are biased because of unobserved variables (25).

A related analysis, which we will relegate to future work, would consider voxel-by-voxel path analysis. A concern with this approach, however, is that the potential mediating volumes are diffuse and may offer little information regarding localization of the mediating effect at the voxel level. The current approach offers a compromise between the rigidity of anatomically based structural volumes and the extreme localization that would be attempted by a voxel-wise path analysis.

<table>
<thead>
<tr>
<th>Domain, association volume, tissue type</th>
<th>Estimate*</th>
<th>SE*</th>
<th>95% CI*</th>
<th>Permuted estimate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visuo-construction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gray matter</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lead</td>
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<td>0.12</td>
<td>0.16, 0.62</td>
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<tr>
<td>Lead</td>
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<td>0.06</td>
<td>0.08, 0.32</td>
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</tr>
<tr>
<td>Domain</td>
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<td>0.07</td>
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</tr>
<tr>
<td>Executive function</td>
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<tr>
<td>Gray matter</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.24</td>
<td>0.08</td>
<td>0.14, 0.44</td>
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</tr>
<tr>
<td>Domain</td>
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<td>White matter</td>
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<tr>
<td>Lead</td>
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<td>0.05</td>
<td>0.07, 0.29</td>
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<td>0.05, 0.26</td>
<td>0.01</td>
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<tr>
<td>Eye-hand coordination</td>
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<tr>
<td>Lead</td>
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<tr>
<td>Domain</td>
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<td>0.04</td>
<td>0.03, 0.20</td>
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<td>White matter</td>
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<tr>
<td>Lead</td>
<td>0.15</td>
<td>0.05</td>
<td>0.07, 0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>Domain</td>
<td>0.14</td>
<td>0.05</td>
<td>0.06, 0.25</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Estimate, estimated proportion of the total effect that is indirect; SE, standard error; CI, confidence interval; permuted estimate, estimated proportion of the total effect that is direct from the permuted data sets.

0.10, 0.15, and 0.14 corresponding to the association volume ordering from top to bottom in table 6, respectively.

The permuted analysis showed little evidence that the suggested mediation was due to chance associations arising from multiplicity concerns. The effect of lead on cognition remained significant after adjustment for the gray matter lead association volumes based on the permuted data in 82 percent, 100 percent, and 100 percent of the simulations for the visuo-construction, executive function, and eye-hand coordination domains, respectively. The results were similar in the remaining settings. In addition, table 6 shows the mean proportion of the total effect that was indirect across the permuted data sets. The estimated proportions were 0.04 or lower, well below the ones based on the actual data. Note that these numbers can be used as benchmarks for the bias in the actual data results. In summary, because the permuted results were not similar to results from the actual data and presented overall weak or no evidence of mediation, we concluded that the (real data) results were not attributable to chance associations.
ACKNOWLEDGMENTS

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Conflict of interest: none declared.

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