Effect of Washing Procedures on Trace-Element Content of Hair

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A pooled sample of hair was divided and portions prepared for analysis by three washing procedures, to evaluate the effect of washing procedure on the subsequent trace-element (Zn, Cu, Mg) content. The methods selected were a detergent wash, a hexane–ethanol wash, and an acetone–ether–detergent wash. For all elements, there was a significant difference among the results after these wash procedures. Magnesium content of hair was most affected by washing, containing less than half of the magnesium of the unwashed hair. The detergent wash removed the most zinc and magnesium; the acetone–ether–detergent wash removed the most copper. Our results indicate that the trace-element analysis of hair is sensitive to the preparation technique and therefore is an unreliable source of information about trace-element status.

Recently there has been a great amount of interest in the use of hair in assessing trace-element status of the body. Numerous authors have indicated that the trace-element content of hair may be related to the corresponding concentrations in the tissues (1–7).

The advantages of using hair as a diagnostic sample is that it is easily obtained and can be stored for long periods of time until an analysis can be performed. More importantly, once incorporated into the hair, the trace elements are no longer in equilibrium with the body and thus are not susceptible to circadian variation. On the contrary, hair samples represent an ill-defined period of time prior to the time of collection. Hair is also susceptible to external contamination from the environment. Therefore, it is essential that hair be washed to remove surface contamination before analysis. Several different methods have been proposed and used for washing. They do not necessarily result in the same final data, but little effort has been made to determine the reliability of the results.

Hilderbrand and White (8) compared different methods; however, their report had an incomplete statistical design and insufficient replicate samples for an adequate evaluation of methodology. Harrison et al. (9) compared a detergent with an organic solvent method. Their studies of methodology were done with no more than three replications, showed large variations between replicates with the same wash method, and there was no statistical evaluation of the data. Therefore, only subjective conclusions could be drawn. The purpose of this

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Table 1. Some Trace Elements in Hair before and after Different Washing Procedures

<table>
<thead>
<tr>
<th></th>
<th>Unwashed</th>
<th>Detergent</th>
<th>Hexane/EtOH μg/g</th>
<th>Acetone/ether/detergent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>17.1 ± 1.1*</td>
<td>13.0 ± 1.9</td>
<td>14.9 ± 1.3</td>
<td>9.4 ± 1.1</td>
</tr>
<tr>
<td>Zinc</td>
<td>172.9 ± 5.6</td>
<td>158.1 ± 5.6</td>
<td>171.3 ± 7.2</td>
<td>168.6 ± 4.4</td>
</tr>
<tr>
<td>Magnesium</td>
<td>78.7 ± 2.1</td>
<td>30.5 ± 3.6</td>
<td>32.9 ± 2.4</td>
<td>34.3 ± 1.9</td>
</tr>
</tbody>
</table>

* Mean ± SD for 10 replicate samples for each washing procedure.

tubes. Concentrated nitric acid, 3 ml, was added to each of the tubes, which were left in a water bath at 90 °C for about 2 h. The then-digested samples were diluted to 20 ml with deionized water and analyzed for zinc, copper, and magnesium by atomic absorption spectrophotometry (Perkin Elmer Model 306, Perkin-Elmer Corp., Norwalk, Conn. 06856). An additional 10-fold dilution was necessary for zinc to attain the appropriate analytical range.

Results and Discussion

The observed concentration of zinc, copper, and magnesium in our hair samples (Table 1) showed that the zinc content was approximately fivefold greater than that of magnesium, and magnesium content was twofold that of copper. These relative values concur with results found for hair in other studies (4, 9, 13).

Chelating agents and dilute hydrochloric acid, used by others, were avoided in this study (8, 14). These agents are known to cause the dissociation of some of the tightly bound trace elements that should rightly remain a part of the hair sample.

Our results show that there was significant decrease in the concentration of trace elements as a result of washing, except for zinc content, after the hexane wash. There also was a significant difference between the various washing procedures for all three elements tested, copper \( (P < .005) \), zinc \( (P < .005) \), and magnesium \( (P < .05) \), although there was a high degree of reproducibility within each of the washing procedures, as shown by the small CV within methods (3–15%). The greatest effect due to washing was shown in the results for magnesium. The concentrations in the washed sample were less than half those in the unwashed sample. Zinc showed the least difference between unwashed and the washed preparations. The detergent wash produced the greatest decrease in concentrations of magnesium and zinc as compared with the unwashed hair samples and was generally the least reproducible. The acetone–ether–detergent method produced the greatest decrease in the concentration of copper. The all-organic hexane–ethanol wash evidently removed the least copper and zinc contaminants. The copper content showed the greatest variability after washing by the different methods, magnesium showed the least. The least total change was that in zinc.

Our results showed that the trace-element content of hair is generally sensitive to washing procedures, the one exception being the zinc content of hair after the hexane–ethanol wash procedure. We doubt that hair can be used as a reliable diagnostic sample; certainly the results obtained by different laboratories utilizing different washing procedures should not be compared.

We did not determine whether or not only those contaminants from environmental sources are the ones that are removed by washing. However, use of hair analysis for epidemiological surveys and diagnostic considerations of trace-element status is technique sensitive and thus may be unreliable.

References