Hair—Metal Binding*

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Extensive ambient extraction of several metals from human hair compromises some assumptions concerning the binding of metals to hair and the biochemical process of metal incorporation into hair. Hair metal concentrations can reveal metal intoxications and metal deficiencies.

The scalp hair selected for investigation included specimens of different colors from both sexes for various donor ages and from different geographic exposure areas. The hair samples were washed by agitation for 30 min with a 1% solution of sodium lauryl sulfate in a pH 7.2 buffer, rinsed repeatedly with deionized water, and oven-dried at 110°C before 2-g portions were taken for extraction and digestion. Extraction involved agitation of the washed hair samples for 21 hr with 40 ml of 1% nitric acid at room temperature (23°C). The extracted hair was digested in order to quantitate the amount of metal that was not extracted. Metal measurements were made by conventional flame atomic absorption spectroscopy. Potential interferences were investigated.

Extraction varied between 82 and 100% for Na, Mg, K, Ca, Mn, Zn, Cd, and Pb. Removal of Fe, Ni, and Cu was 33–54%. Anatomical location, binding chemistry, biochemical incorporation, and mass screening are discussed.

The analysis of human hair for its trace content of metals continues to attract interest as a monitor of excessive or inadequate ingestion of various metals (1–19). Limitations in such studies have been the subject of some debate (15, 17–30). Metal-to-hair binding has been considered to be so intimate or tenacious for endogenous metals that only total digestion would release the metals into solution. In most reports this opinion has been expressed only implicitly, but one recent publication (16) contained the assertion that "...a variety of heavy metals...are firmly bound" to hair. One group of established investigators (26) has raised the possibility "that many essential trace elements are required...for the structure of the keratinized hair shaft." External contamination has been invoked to account for the proportion of metals removed from hair by washing treatments (15, 16, 24, 31, 32). Evidence obtained in our laboratory is not fully consistent with these opinions.

Metal Extraction and Analysis

Ambient extraction of hair samples with 1% nitric acid has been found to remove an average of 84% or more of several metals and at least 33% of certain others. The hair samples were washed with a detergent solution before the extractions, and subjected to total digestion after the extracts were decanted. The hair samples showed no visible changes as a result of the ex-
traction treatment (Fig. 1) and retained 96% or more of their initial dry weights, indicating that hydrolysis (if any) was not extensive. In Figure 1 the dark shading attributable to pigmentation is similar for the two fibers. In addition, both fibers exhibit the unpigmented surface cell layer. Potential interferences and background absorption were investigated. While a practical use for the ambient extraction of hair samples for mass screening of some metals is indicated by the evidence reported, it was not the purpose of this study to determine the optimum reagents or conditions for the ambient extraction of metals from hair. Considering prevailing opinions to the contrary, reporting extensive extractability of hair metals by any agent plus the binding implications was considered adequate justification for this report.

Table 1 shows the results obtained by atomic absorption spectroscopy when ten washed hair specimens were soaked in 1% nitric acid for 21 hr at room temperature (23°C) before the hair was digested. The initial metal concentrations for the washed hair specimens were determined by combining the paired extract and digest measurements. The scalp hair selected for this study included specimens of different colors from both sexes for various donor ages (from 12 yr to 60 yr) and from different geographic exposure areas in an effort to obtain a set of hair samples representative of the general population.

All containers were acid-cleaned by use of concentrated nitric acid. Hair samples were washed by agitation for 30 min with a 1% solution of sodium lauryl sulfate in a pH 7.2 buffer, rinsed repeatedly with deionized water, and oven-dried at 110°C before 2-g portions were taken for extraction and digestion. For the extraction treatment, hair samples were submerged (by use of a clean glass rod) in 40 ml of 1% nitric acid and agitated on a mechanical shaker. After 21 hr, the extracts were decanted and the hair pressed with a glass rod to remove as much fluid as possible. The extracted hair was digested by sequential additions of concentrated nitric acid (20 ml X 2) and perchloric acid (2 ml) according to similar published procedures (14-16, 32). The digest volumes were evaporated to 4 ml or less before the final digest volumes were adjusted to 40 ml with 1% nitric acid.

Metal measurements were made by conventional flame atomic absorption spectroscopy with calibration against commercial aqueous standards. Since additions of standards to portions of both the extracts and the digests gave full recoveries indicating no interferences, calibration by the method of standard additions was not necessary. Other investigators (33) have found no interferences in the atomic absorption analysis for four of these metals in hair after complete digestion in acid, although application of the method of standard additions seems redundant after demonstrating that interference was absence, and a discussion in this same report (33) on ionization is open to serious criticism (34). It was necessary to add an excess of sodium (1000 μg/ml) to the potassium standards to suppress ionization losses. Tests indicated that adding sodium to the sample solutions was not needed. Calcium remeasured after 40-fold dilution in 1% lanthanum chloride solution (to inhibit potential compound formation by calcium) showed the same trend as measurements without added lanthanum chloride. Since nonatomic absorption is not discernable by recovery studies nor corrected for by the method of standard additions (35); measurements for the metals were repeated with use of a deuterium background corrector. The solutions showed the same trends with the background corrector in use although an in-

**Figure 1.** Composite view of two human hair fibers before and after metal extraction.
Table 1. Extraction of metals from human scalp hair.

| Sample | Sample | Sample | Sample | Sample | Sample | Sample | Sample | Sample | Ext. Average (%) | Std. Error |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|-----------------|------------|
| 1a     | 1b     | 2      | 3      | 4      | 5      | 6      | 7      | 8      |                 |            |
| Sex    | F      | F      | F      | F      | M      | M      | M      | M      |                 |            |
| Age    | 55     | 55     | 33     | 43     | 12     | 50     | 60     | 25     |                 |            |
| Sodium |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 2240  | 2220  | 3420  | 1400  | 500   | 710   | 680   | 690   | 3000            | 720        |
| Extracted, % | 95    | 96    | 94    | 94    | 96    | 91    | 97    | 96    | 94              | 97         |
| Magnesium |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 128   | 166   | 24    | 40    | 12    | 31    | 52    | 15    | 48              | 32         |
| Extracted, % | 88    | 92    | 75    | 70    | 100   | 87    | 85    | 100   | 83              | 69         |
| Potassium |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 100   | 120   | 180   | 80    | 20    | 42    | 40    | 30    | 200             | 40         |
| Extracted, % | 100   | 100   | 100   | 100   | 100   | 100   | 100   | 100   | 100             | 100        |
| Calcium |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 1836  | 1706  | 3192  | 974   | 364   | 559   | 288   | 450   | 4056            | 256        |
| Extracted, % | 93    | 95    | 93    | 89    | 92    | 91    | 94    | 97    | 94              | 91         |
| Manganese |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 0.8   | 0.6   | 0.9   | 1.0   | 1.4   | 1.7   | 4.0   | 2.1   | 2.2             | 0.2?        |
| Extracted, % | 75    | 67    | 67    | 100   | 100   | 100   | 95    | 86    | 91              | 50?         |
| Iron    |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 14.6  | 14.0  | 11.7  | 9.8   | 6.6   | 8.2   | 22.4  | 87.0  | 12.8            | 6.4         |
| Extracted, % | 74    | 81    | 64    | 51    | 36    | 36    | 37    | 72    | 52              | 44         |
| Nickel  |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 0.8   | 2.2   | 15.6  | 3.8   | 1.2   | 6.9   | 2.2   | 1.2   | 4.6             | 1.8         |
| Extracted, % | 83    | 73    | 75    | 74    | 33    | 15    | 36    | 36    | 57              | 44         |
| Copper  |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 10.8  | 11.0  | 13.2  | 11.4  | 9.4   | 11.1  | 22.2  | 17.7  | 20.4            | 11.2        |
| Extracted, % | 37    | 34    | 75    | 74    | 33    | 15    | 36    | 36    | 57              | 27         |
| Zinc    |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 151   | 164   | 110   | 181   | 188   | 208   | 221   | 195   | 429             | 169        |
| Extracted, % | 92    | 94    | 92    | 88    | 90    | 91    | 92    | 78    | 94              | 84         |
| Cadmium |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 1.2   | 1.0   | 1.2   | 1.4   | 2.6   | 2.3   | 1.6   | 6.9   | 2.2             | 0.6         |
| Extracted, % | 100   | 100   | 100   | 71    | 92    | 73    | 100   | 83    | 73              | 100        |
| Lead    |        |        |        |        |        |        |        |        |                 |            |
| Concn, μg/g | 22    | 10    | 15    | 12    | 26    | 27    | 106   | 141   | 6               | 2          |
| Extracted, % | 91    | 100   | 80    | 80    | 85    | 85    | 92    | 87    | 100             | 82         |

creased measurement variability occurs when operating the instrument in this single beam mode (resulting from instability in the light emissions from the two lamps). Even without the background corrector, 100 μg/ml solutions of sodium, potassium, calcium, and magnesium standards did not show significant absorption at the analytical wavelengths used for the other elements. At the wavelengths for cadmium and zinc, even 1000 μg/ml sodium did not show significant absorption.

Sodium, calcium, and zinc were measured with the atomic absorption burner at 90° to the light beam to allow measurements on the
original solutions without the need for dilution. For the same reason, potassium and magnesium were measured at wavelengths providing less than maximal sensitivities. Measurements for all the metals were made on acid blanks for both the extracts and the digests as well as on the detergent solution used to wash the hair samples. Corrections were made for the significant levels of copper and iron in the digest acid mixture.

For two of the hair specimens a second portion was extracted, digested, and analyzed as shown in Table 1. The metal values for the two portions agree except for cadmium, lead, and nickel. The lack of agreement for some metals in different portions of hair is not surprising, considering the concentration variation reported (14, 26, 37, 38) to occur at different locations along the same hair shafts. The spread in the hair metal concentrations is not unusual, and can arise from variations in diet, environment, hair treatment, donor age, and sex (1-5, 8-15, 17-33, 37, 38). In spite of the limited sample size, a sex-related factor appears to be operative in the concentration data for sodium, potassium, and calcium (female > male). Since the concern in this report is the extraction of metals from hair, the proportions removed are the pertinent issue, and sample homogeneity is irrelevant. Replicate metal agreement within about ±10% has been reported for fully digested hair specimens (15, 24, 31, 33). Extraction has been achieved with more dilute nitric acid and with a nonoxidizing acid (HCl). Basic pH was not investigated because the protein disassociation that can occur (39) could interfere with the analysis or the interpretations.

"Surface" Binding

It is apparent from Table 1 that many metals associated with hair are removed almost completely by ambient extraction, indicating that most of the binding for these metals must be on the surface that is accessible to liquids (a functional definition for "surface" that includes both external and internal regions). Figures 2 and 3 illustrate the shingled character of hair surface which offers increased area for binding and access for fluid penetration. While the metals showing lower extractability in these tests could be interpreted as reflecting the extent of internally inaccessible metal binding, another interpretation does not require this assumption. These metals could also be largely surface-bound but more resistant to removal. Support for this view is provided by the data in Table 1.

The metals in Table 1 include at least one member from nine chemical groups of the Periodic Table and four chemical periods. All the metals showing extractability below 80% to 1% nitric acid solution form transitional ions possessing a partially filled 3d electronic subshell as indicated in Figure 4. A dramatic increase in extractability (from 33% to 89%) is
observed between the adjacent metals copper and zinc, whose divalent ions differ electronically in possessing an unfilled and a filled 3d subshell, respectively. A filled electronic subshell is well known to contribute extra chemical stability, but a half-filled subshell also possesses extra stability (60, 40a). The divalent manganese ion possesses a half-filled 3d electronic subshell and, unlike its neighboring transitional elements in the periodic table, shows an acid extractability above 80%. Manganese exhibits a reluctance to form organo-metallic derivatives (60). The ammonium pyrrolidine dithiocarbamate (APDC) complex of manganese is unstable (41–43), while the nickel complex is stable (43). The greater extractability of manganese relative to the other transitional metals in hair is consistent with the view that hair “surface” binding could be the major site for all the tested metals. In addition, the binding affinity of hair for the metals tested exhibits the same order as that observed with a variety of soluble ligands (61), i.e. Ca < Mg < Mn < Fe < Ni < Cu < Zn. The stronger the affinity of hair for a metal species the lower the proportion extracted. Hambidge et al. (24) have reported a 57% removal of chromium from hair by a commercial bleaching agent; this is consistent with the predominance of binding to the accessible hair surface for this transitional metal and discounts to some degree the notion that chromium is required for the keratinized hair shaft (26). In addition surface-bound metals on hair (added by soaking) are known to be generally resistant to removal, especially copper (20, 21, 24, 25, 38, 44a). The fraction of copper on hair known by tests to be extractable by 1% nitric acid can be made unextractable by treating the hair with a mercaptan (T. Hinners, unpublished observation). Evidence that zinc and arsenic are localized to the external surface region of the hair shaft is available (44b). Spectroscopic surface examination of the hair shafts should clarify the issue of metal localization. While it is possible that other evidence may implicate significant metal binding in hair for some elements, nonextraction by 1% acid does not appear to provide a reliable distinguishing test.

**Removal of Metals by Washing**

Some form of laboratory washing for hair samples has been considered necessary to remove metal-containing dirt and fluids added by nature or by hair treatments. The metals removed by the washing solutions have usually been considered to reflect only externally added metal (15, 16, 24, 31, 32, 38). From the evidence presented in Table 1, this opinion would appear to deserve reevaluation as a generalization since washing solutions constitute a form of ambient extraction. After demonstrating (26) that chromium contamination of washed hair is a rare occurrence, Hambidge et al. (24) nevertheless ascribed the 29% chromium removal from hair by washing to removal of external contamination. The same authors reported (24) that chromium added to hair by
soaking was only removed to the extent of 3% and 55% in two cases by a single washing treatment and even repeated washing did not remove all the added chromium. From extraction tests with 1% nitric acid on washed hair sampled (T. Hinners, unpublished data), it is apparent that hair specimens differ dramatically in the rate at which they release metals. Soluble proteins also show very different rates for the release of Zn (64). Harrison et al reported (92) a difference in iron and magnesium removal from hair by washing with detergent compared to washing with a mixture of organic solvents; but these authors were uncertain whether this finding represented a differential removal of exogenous or endogenous metal.

**Binding Sites**

The binding of metals to the hair shaft is considered to involve sulfur (14, 16, 27, 28). The human hair shaft is principally protein in nature (39a) and contains between 11 and 18% of the sulfur-containing amino acid cysteine and its dimer cystine (39a, 45). A small amount of methionine sulfur is also present (39a, 45). It should be noted that hair shafts above the skin layer are metabolically inactive and contain a layer of cuticle cells over the cortical cells, which form the bulk of the hair shaft, plus a central core of medullary cells (39a, 44b, 46a). The cells in the hair shaft are not just inactive but degenerate, since they have lost much of the subcellular components present below the skin layer (39b, 46a). The width of scalp hair range between 25 and 125 μ according to one source (47).

In view of the tenacity attributed to metal-sulfur bonding (14, 16), some investigators may consider the extractability of metals from hair to be inconsistent with binding to sulfur. However, metal–sulfur bonds as a class are not unusually stable (48) as evidenced by the well-known reaction (40b) between zinc sulfide and dilute acid (with the apparent release of hydrogen sulfide) as well as by the use of zinc to reduce organic disulfides to monosulfides (49a), the use of barium hydrosulfide in the laboratory synthesis of cysteine (49b), and the ferric-catalyzed oxidation of cysteine (at basic pH) to cystine (49b, 50). Evidence of zinc-sulfur binding and release of zinc in acid has been reported for soluble proteins. The high stability of the mercury–sulfur bond (40c) is the exception rather than the rule for many metal sulfides. The influence of a mercaptan to decrease extraction of copper (but not zinc) from hair would seem to involve stable copper-sulfur bonding. Cupric sulfide is similar to mercuric sulfide in its slight degree of dissociation, and a few tests have indicated that mercury is not removed from hair by 1% acid. Silver sulfide is also slightly disassociated, and Bate (20) has reported only meager removal of externally added silver from hair. Additional tests may clarify whether the extractable hair copper was nonsulfur-bound or weakly associated with sulfur in the hair.

Carboxyl groups may play a significant role in the binding of metals to hair since it has been reported (20, 24) that hair adsorbs more metals at pH 6 than at pH 4. Unbound carboxyl groups would be more protonated at lower pH and would present fewer carboxyl anions for binding with the metal cations. Extraction of metals by 1% nitric acid (pH 0.9) is consistent with this trend for metal binding to decrease with decreasing pH, but does not require only carboxyl involvement, since, as noted above, some metal–sulfur bonds could be affected. While the α-carboxyl groups of amino acids are bound in amide formations in the protein polymers, hair proteins consist of about 19% of the dicanoxyl amino acids glutamic and aspartic acids (45). Some dipole bonding to serine hydroxyl groups and to certain nitrogen groups is also feasible. Interestingly, anionically charged nonmetals have been reported (20) to bind more to hair at lower pH (when more anionic carboxyl groups on hair would be neutralized by protonation).

**Evidence for Extraction of Metal from Hair**

Ambient extraction of metals from hair has been reported before. In a 1966 report (20) on a study largely concerned with the adsorption and elution of isotopic elements, Bate reported extensive removal of “natural” zinc by ambient extraction of hair with a chelating agent. In the determination to reject his conclusion that external contamination may explain the origin of many of the elements observed in the hair,” some investigators (15, 22, 24) apparently ig-
nored this experimental finding. Bate's conclusion is inappropriate, since radioisotope injections (23, 51, 52) as well as deficiency-supplementation studies (1-5) have established that ingested metals do reach the hair. Schroeder and Nason (21) cited Bowen in a footnote in reporting that detergents remove many trace metals successively from hair. Harrison et al. (32) have cited Perkons and Jervis in reporting that soaking of hair in distilled water, ionic detergent, or a 50/50 mixture of acetone and alcohol for 40 hr results in a change in the concentration of trace elements in the hair. In addition, significant removal of iron and magnesium from hair by detergent washing but not by a mixed organic washing was reported (32).

Water, organic solvents, detergents and acids are known to denature proteins (53a), and differences among these agents in this property may account to some extent for the differences in removal of metal from hair. Hair can be converted from the α-keratin to the β-keratin structure in water (53b). Interestingly, native egg albumin (a protein) contains no detectable -SH groups, but upon denaturation the number of -SH groups detectable is equal to the number after complete hydrolysis with strong acid (53c). The reported resistance of hair lead to removal by dilute acids (14) could be due to insufficient contact time.

It is apparent that many metals are not firmly bound to hair exposed to 1% acid and that of these metals most are not required for the gross structural integrity of hair. "Firm" binding can occur at pH 6; the possibility that portions of some metals are structurally necessary to hair has not been eliminated entirely.

**Biochemical Implications and Speculations**

While incorporation of metals into (or onto) hair may occur in the hair bulb, some role for a secretory mechanism below or at the skin surface is difficult to reject. If ambient exposure to fluid can remove some metals significantly, then the reverse is conceivable under physiological conditions; this does occur in vitro, as indicated by soaking studies (20, 24, 25, 38, 44a). The localization of zinc and arsenic to the external surface region of hair (44b) is consistent with addition via secretion. It may be relevant that the hair cuticle layer retains sulfhydryl groups longer than the cortex (54b). Metal incorporation may occur directly at the keratogenous zone above the hair bulb, since radioactive cystine seems to be taken up directly at this zone in mice (55). It could be significant in this latter respect that cystine is found (along with other amino acids) in sweat (56). Secretion could involve sweat glands and sebaceous glands. Although sweat has been discredited as a factor in hair metal content (15, 22, 24), a significant loss of some trace metals has been cited (29), as sufficient to exceed daily intake (at least for zinc). Some athletes (known to sweat) have been reported (57) to show a low zinc status. Sebaceous glands are attached to most hair canals just below the skin surface (46b) where the fully keratinized hair shaft receives the sebum secreted by the glands. The report that 210Pb accumulates in growing hair more than in nongrowing hair of the rabbit (58) is not definitive support for growth incorporation, since both the hair growth and the sebaceous glands may be inactive (44c, 46b) in nongrowing hair roots (follicles).

In support of some role for a secretory mechanism are reports concerning the appearance of injected metal isotopes as well as nonisotopic metals associated with the outer hair after days (44b, 52) and even after just 2 hr (23) or 4 hr (38). Hair is reported to grow at a rate of 0.2-0.4 mm/day (14, 26, 44a, 59). Some form of migration on the hair shaft might be involved if this latter evidence is given credence. The existence of metal concentration gradients along hair shafts does not support the idea of unlimited migration. While some of these biochemical speculations may prove to be incorrect, they are presented as possibilities consistent with current knowledge.

**Summary**

Extensive removal of several hair metals by ambient extraction with 1% nitric acid is documented for a variety of hair specimens. Anatomical location and chemical nature of the metal binding to hair are discussed with reference to other published reports. Secretion of metals onto the preformed or nearly formed hair shaft is consistent with the evidence ob-
tained, at least for a majority of the metals tested. Ambient extraction of some hair metals could be considered acceptable for mass screening purposes, since total digestion procedures are reported to have a metal replicate variability of about ± 10%.

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