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The Spatial Distribution of Zinc and Cobalt in Single Human Head Hairs

In a previous publication [1], which contained a full bibliography, data were presented on the concentration patterns of copper in individual human head hairs. Concentration fluctuations were observed between adjacent 2-mm long segments of the hair shaft, while for many subjects there was a general increase of concentration from the root to the distal end of the hair plus local regions of increased and decreased concentration.

Further experiments, in which hairs were soaked in solutions of cupric ions, showed that copper taken up by the hair exhibited very similar concentration patterns. This supported the conclusion that most, if not all, of the indigenous copper observed in the hairs studied arose from uptake from the hairs' environment (that is, sweat or external contamination) and that concentration fluctuations arose from variations in absorption capacity for copper. Zones of increased capacity were evidently fixed in the hair structure and receded from the scalp as the hair grew. It was considered of some interest to extend such studies to other elements. The present paper deals with zinc and cobalt, elements which, like copper, are essential to human biochemistry and which also exist in aqueous solution as hydrated divalent ions.

Zinc is of particular interest since the zinc content of hair is often the largest for the metallic elements present and is known to be a sensitive index of the level of zinc in the body and hence of zinc deficiency [2]. Further, in an earlier study [3] of 10-cm segments of the hairs of Subject A, also studied in this work, zinc alone among nine elements studied exhibited a concentration almost invariable from one end of a 50-cm long hair strand to the other. This is in stark contrast to the strongly varying copper concentrations observed in hairs from the same subject [1].

Experimental Technique

Measurement of Natural Zinc Content

The experimental procedure was generally similar to that employed earlier for copper [1]. Individual hairs were plucked, with precautions to avoid contamination, verified by microscopic examination as being in the anagen phase of the growth cycle, and washed via a previously described water-acetone-ether cycle, but with the washing time increased from 5 to 10 min per step. This was necessary to reduce erratic fluctuations in the measured zinc content of adjacent 1-mm hair segments, perhaps due (as in the case of copper) to superficial contamination.

The hairs were cut, as before, into segments under microscopic observation, and the

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zinc content measured by flameless atomic absorption (AA) spectrometry. A Perkin-Elmer Model 305 spectrometer equipped with a Model HGA-70 graphite furnace was employed; the hair sample was dried for 10 s at 100°C, ashed for 15 s at 1100°C, and then the zinc atomized at 2200°C. Care was taken to place the hair sample at the center of the furnace initially, and visual observation confirmed that it was not displaced by the argon filler gas stream during measurement.

Calibration was achieved by injection of aliquots of an aqueous solution prepared from known masses of zinc metal dissolved in nitric acid. Validity of the analyses was occasionally checked by addition of nitric acid to dissolve the residue left after ashing of a hair sample. Repeating the drying and ashing steps then resulted in atomization of zinc from a chemical form very similar to that existing during the calibration procedure. No change was observed as a result in the measured zinc values beyond the fluctuations observed in any case between adjacent segments from a given hair shaft.

The sensitivity of the flameless AA procedure for zinc is such that the zinc content was easily measurable in segments of length down to 0.5 mm, the limit with conventional manipulative techniques. The measurement precision is estimated at $\pm 10\%$.

The sensitivity of the technique for cobalt is inadequate for measurement of the (much lower) natural cobalt content of hair segments of several centimetres in length. Thus no information on this topic could be added to that contained in an earlier paper [3] for hairs from Subject A, which showed (unlike zinc) a general increase of cobalt concentration with increasing distance from the hair root.

Radiotracer Techniques

Zinc-65 and cobalt-60 radiotracer solutions were obtained from New England Nuclear Corp., Boston, Mass., and had reported specific activities of 3.7 and 0.1 mCi/mg, respectively. The zinc concentration and pH of the first solution were adjusted to 0.1 mg/ml and 5.5, respectively, while the cobalt concentration and pH of the second solution were adjusted to 0.3 mg/ml and 8.5, respectively. At this pH, the cobalt solution did exhibit some precipitation of cobalt hydroxide; this pH value was, however, maintained in the expectation [4] (confirmed by observation) that radiocobalt uptake was more intensive than at lower pH values, the precipitation notwithstanding.

After soaking in these solutions for known lengths of time, the hairs (except those specially treated for the experiment of Fig. 12) were rinsed successively in water, acetone, and ether, each for 1 min, and then cut into 2-mm segments by the aforementioned technique, each segment being mounted on an aluminum planchet for radioactivity assay. This was achieved by means of a Si(Li) beta detector coupled to conventional electronic apparatus.

Conversion of the measured radioactivity to mass of zinc or cobalt taken up was achieved by measurement periodically during the experiment of samples prepared by evaporation of aliquots of the radiotracer solutions described above, under counting geometry and other conditions identical to those under which the hair samples were measured.

Results

The five female subjects from whom hair samples were examined in this study are described in Table 1.

Indigenous Zinc Content

Figure 1 shows the zinc patterns observed for two hairs from Subject B, and Fig. 2 shows the patterns for two hairs from Subject I. Aside from a high zinc content immedi-

TABLE 1—Subjects from whom hair samples were studied. All subjects were residents of Vancouver, British Columbia.

Subject	Age	Sex	Hair Color
A	21	female	light brown
B	43	female	dark brown or black changing to white or gray
G	22	female	brown, partially bleached
I	25	female	dark brown
J	29	female	light brown, partially bleached

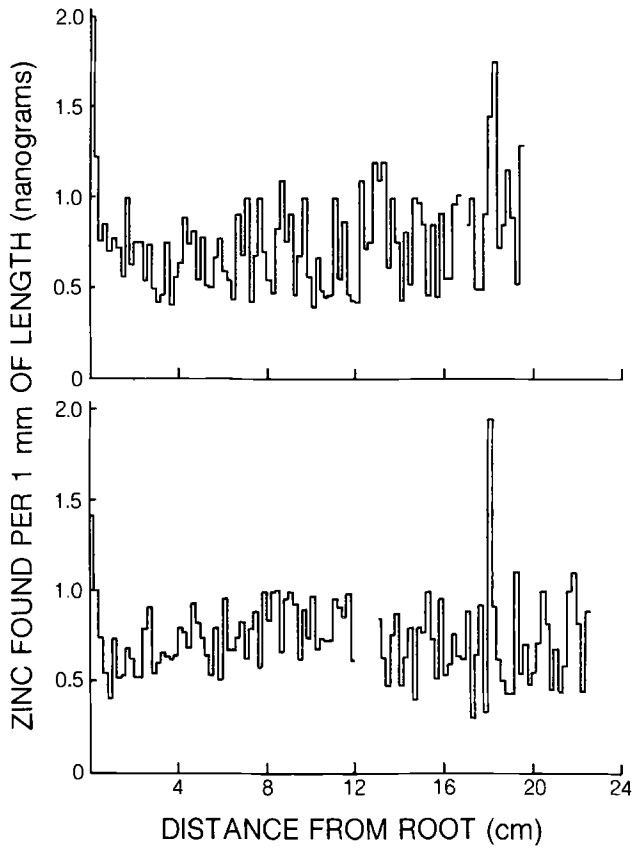


FIG. 1—Indigenous zinc content of 1-mm segments of two hairs from Subject B.

ately adjacent to the root and substantial segment-to-segment fluctuations, the data indicate zinc contents otherwise substantially constant from one end of the hair to the other. This behavior is essentially identical to that observed for the zinc patterns (observed at much lower resolution) for Subject A in an earlier study [3], and in marked contrast to that observed previously [1] for copper in the hairs of Subjects A and B.

In view of the increased copper content observed earlier [1] in hairs subjected to a cosmetic bleaching procedure, it was considered of interest to examine such hairs for their zinc content. The top curve of Fig. 9 shows the indigenous zinc pattern observed in a hair from Subject J, which exhibited two zones of decoloration, one between about

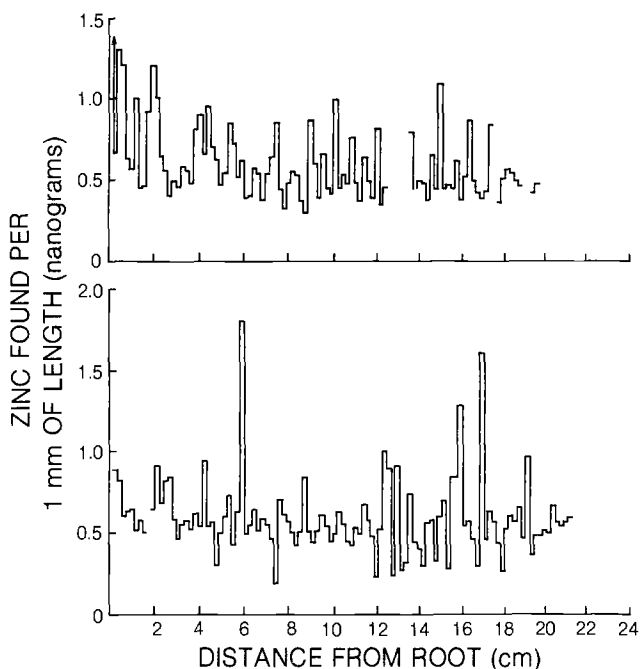


FIG. 2—Indigenous zinc content of 1-mm segments of two hairs from Subject I.

5 and 10 cm from the root and a second from 12 cm onwards. It is observed that the zinc content in the bleached regions is *decreased* by about a factor of four, compared to that in the unbleached hair regions nearby.

Zinc Uptake

In view of the dissimilarity of the zinc and copper concentration patterns, it was also considered of interest to attempt (as earlier in the case of copper) to determine the origin of the zinc observed in hair.

Hairs from several subjects were therefore soaked in the above radiozinc tracer solution and the zinc uptake (in amounts typically ten times the indigenous zinc content) determined by radioassay as described earlier. Figure 3 shows the patterns observed in three hairs from Subject A; Fig. 4, two hairs from Subject B; and Fig. 6, three from Subject I.

In contrast to the flat patterns observed for the indigenous zinc content in hairs from all three subjects, the patterns for added zinc show (in addition to segment-to-segment fluctuations) an increasing uptake with increasing distance from the root plus local zones of increased concentration, features very reminiscent of those observed in the patterns for copper, both indigenous and added. In the case of the copper uptake experiments, increased time of exposure to the cupric ion solution resulted in a higher concentration of copper taken up, and no evidence of saturation of the hair with copper. Figures 4 and 5 show the same behavior in the case of zinc.

In the case of copper, zones of locally increased copper concentration and other pattern features were observed to occur at about the same distance from the root in anagen hairs plucked from a given subject at the same time. Figures 3 and 6 contain evidence that the same is true for zinc for Subjects A and I. Increased concentrations of zinc are observed in all three hairs in Fig. 3 at about 1.5 cm and between 8 and 12

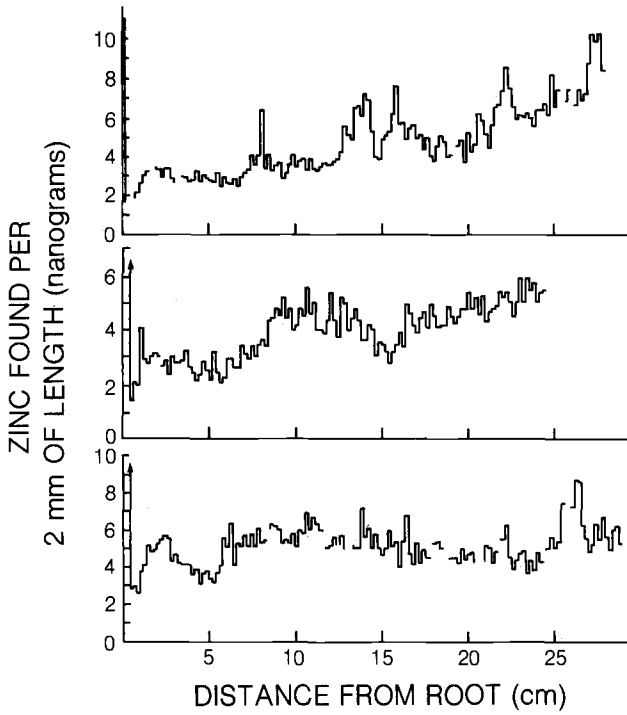


FIG. 3—Zinc absorbed by three hairs from Subject A from a radiozinc tracer solution with a soaking time of 24 h.

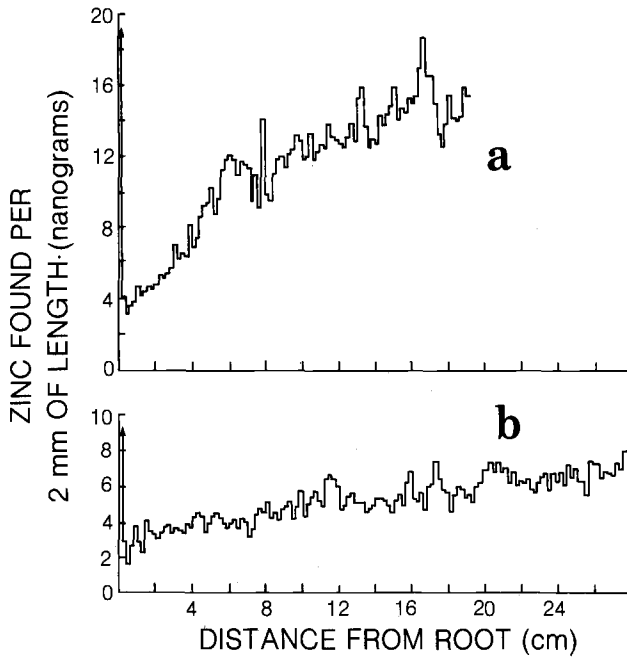


FIG. 4—Zinc absorbed by two hairs from Subject B from a radiozinc tracer solution with a soaking time of (a) 42 h and (b) 4 h.

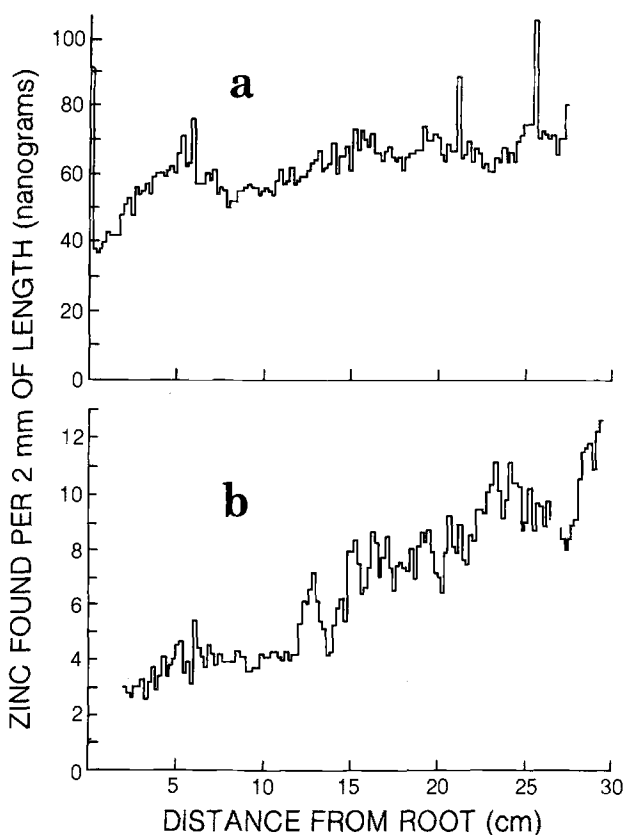


FIG. 5—Zinc absorbed by two hairs from Subject A from a radiozinc tracer solution with soaking times of (a) 78 h and (b) 2 h.

cm from the root, and in Fig. 6 a steeply sloping pattern becomes abruptly flatter at roughly 10 cm from the root. Figure 7 suggests, however, that, for Subject A, zones of increased zinc concentration (near 5 and 15 cm from the root) occur at positions different from those for zones of increased copper concentration (near 10 and 20 cm from the root) for anagen hairs plucked at the same time.

Copper uptake patterns in hairs previously subjected to a bleaching procedure were previously found to resemble patterns for indigenous copper [1], the bleached hair regions exhibiting an elevated concentration in both cases. The results of a zinc uptake experiment on a hair (bleached from 8 cm outwards from the root) from Subject G in Fig. 8 and on a hair (with the two bleached regions described earlier) from Subject J in Fig. 9 indicate that higher zinc uptake does indeed occur in bleached hair. However, the data in Fig. 9 (for two hairs plucked simultaneously) indicate in rather striking fashion that high uptake of zinc in bleached hair regions is the counterpart of the sharply reduced indigenous zinc content of the same regions referred to above.

The radiocobalt tracer experiments, the results of which are shown in Figs. 10–12, resulted in much less cobalt being absorbed from solution than either copper or zinc under comparable circumstances, corresponding perhaps to the much lower indigenous cobalt content. Only in the bleached regions of the hairs of Subjects G (Fig. 10) and J (Fig. 11) was substantial uptake observed, the difference between bleached and unbleached zones being somewhat less clear-cut than in the case of copper or zinc uptake. The data of Fig. 12 indicate further that the cobalt which is taken up by bleached hair is rather easily

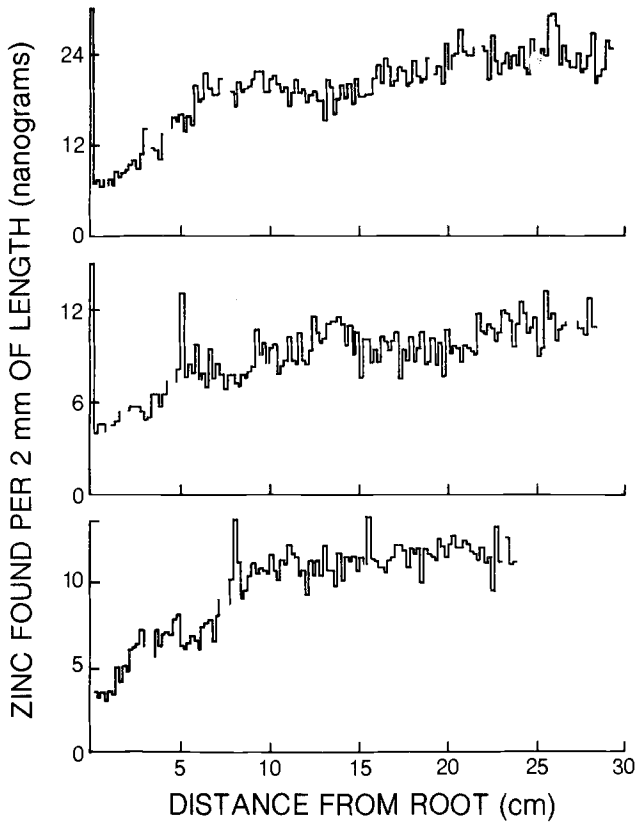


FIG. 6—Zinc absorbed by three hairs from Subject 1 from a radiozinc tracer solution with a soaking time of 136 h.

removed by washing with water. All these patterns show similar segment-to-segment fluctuations to those observed in copper and zinc patterns.

Discussion

The results of the radiozinc tracer experiments reveal a behavior of hair for absorption of zinc from solution very similar to copper absorption, except for the indication that local zones with an increased capacity for zinc uptake are found at positions on the hair shaft different from those for copper. Such behavior may reflect a variable density on the hair structure of binding sites for metal ions, the nature of the sites differing to some extent for copper and zinc. Variations in the density of, for example, cystine along the hair shaft (the disulfide linkage in cystine having been shown [5] to be important for keratin cohesion) might be expected to be accompanied by an increased density of $-SH$ groups, and the copper-sulphur bond has been shown to be stronger than the zinc-sulphur bond [6].

In addition, the rate of diffusion of metal ions into the hair structure (revealed for zinc by the data in Figs. 4-6) may also vary from place to place on the hair shaft and play a role in determining the concentration pattern of metal ultimately absorbed. This may be particularly important in the case of bleached hair; bleaching results in a degradation (and increasing porosity) of the keratin structure, as well as a probably in-

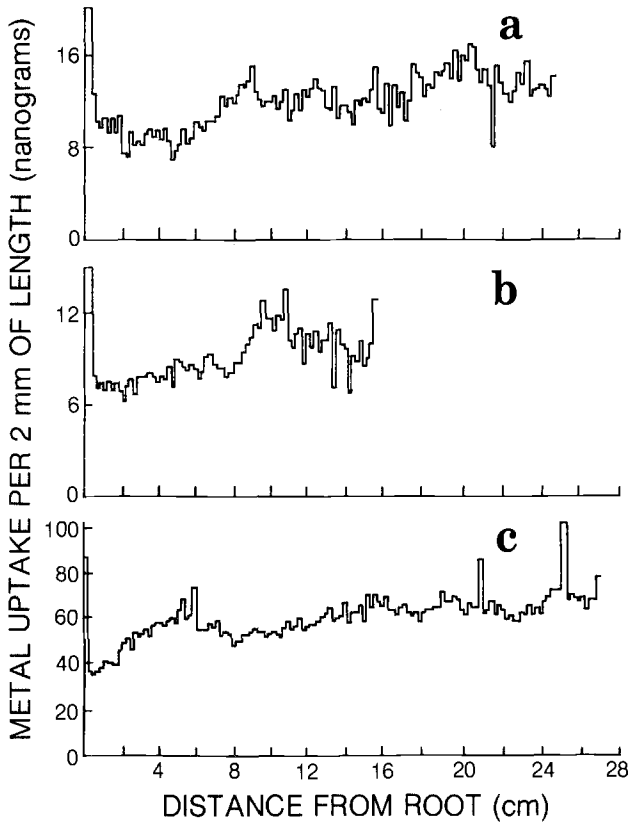


FIG. 7—Metal absorbed by three hairs from Subject A from radioactive tracer solutions: (a) and (b) copper (data from Ref 1) and (c) zinc.

creased density of sulfide metal-binding sites resulting from disulfide bond cleavage. Both could influence copper and zinc absorption.

The data from the radiocobalt tracer experiments suggest a lower density of cobalt-binding sites or a lower diffusion rate of cobalt ions into the hair structure (or both), which, together with the difference of cobalt from zinc in complexing properties, may also account for the low indigenous cobalt content of hair.

There remains the phenomenon of the flat patterns observed for indigenous zinc in unbleached hair characteristic at least of certain subjects living in this area, and the *reduced* indigenous zinc concentration observed in bleached hair from Subjects G and J. The contrast with the patterns observed for indigenous copper on the one hand and absorbed zinc on the other is consistent with the hypothesis that the indigenous zinc patterns observed in this work did *not* originate (as did perhaps the indigenous copper patterns) from absorption by the hair of metal from external sources such as sweat or environmental contamination. This would leave incorporation of zinc at a constant rate into the growing hair shaft from the bloodstream via the follicle as a possible interpretation of the data. The decreased zinc content of bleached hair would then be interpreted as arising from a loss of the originally incorporated zinc, because of the increased porosity induced in the hair by bleaching, and consequently the increased accessibility of the zinc to leaching during hair washing. The segment-to-segment fluctuations observed in all the indigenous and absorbed metal patterns measured might also result from natural,

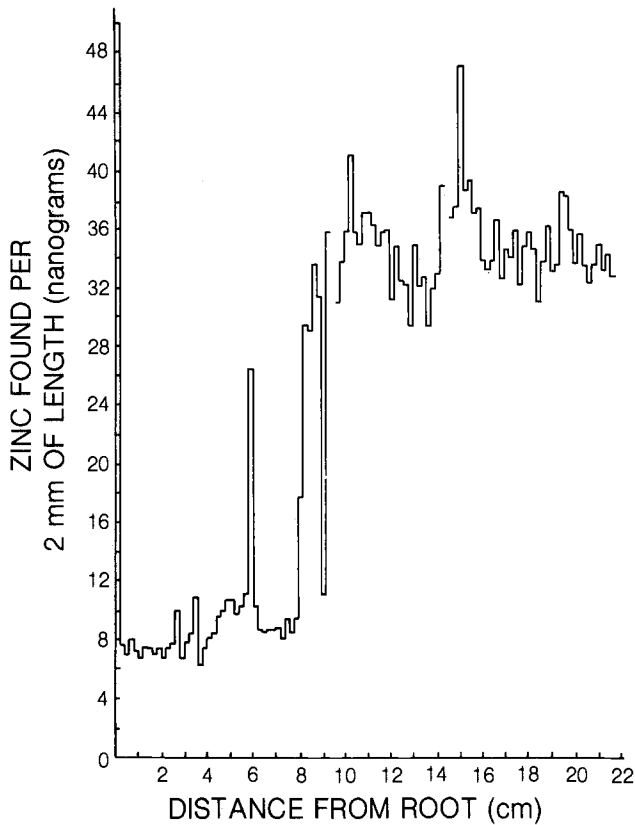


FIG. 8—Zinc absorbed by a hair from Subject G from a radiozinc tracer solution with a soaking time of 1 h. Hair showed a bleached region from 8 cm from the root onwards.

smaller-scale variations in porosity, leading to localized variations in leaching of indigenous metal or absorption of added metal.

It follows from the above hypothesis, and the observation of an inclined structured concentration pattern following soaking of hairs in zinc ion solutions, that the flat patterns observed in this work arose because the contribution to the total zinc content of the hair from external contamination was small compared with incorporation of dietary zinc via the follicle. It follows that in hair from areas where zinc levels in the environment were higher, inclined structured patterns for indigenous zinc would be expected. It is perhaps significant that such patterns have been observed in the hair of women in India [7] (that is, the part of India in question may be one such area).

Otherwise, and in particular for hairs such as those examined in this work, the featureless patterns for indigenous zinc would seem to be of limited value for forensic purposes, such as hair matching. On the other hand, the absorption patterns revealed, for example by radiotracer soaking experiments with zinc, (particularly if combined with like measurements involving, for example, radiocopper) appear to be of considerable potential value for hair matching, given the similarity of patterns observed for hairs plucked from a given subject at the same time.

A difficulty exists in the fact that many hairs encountered in a forensic context are in the telogen phase of the growth cycle, while the present observations are all for anagen hairs. However, the patterns in telogen hairs are expected to be similar to those

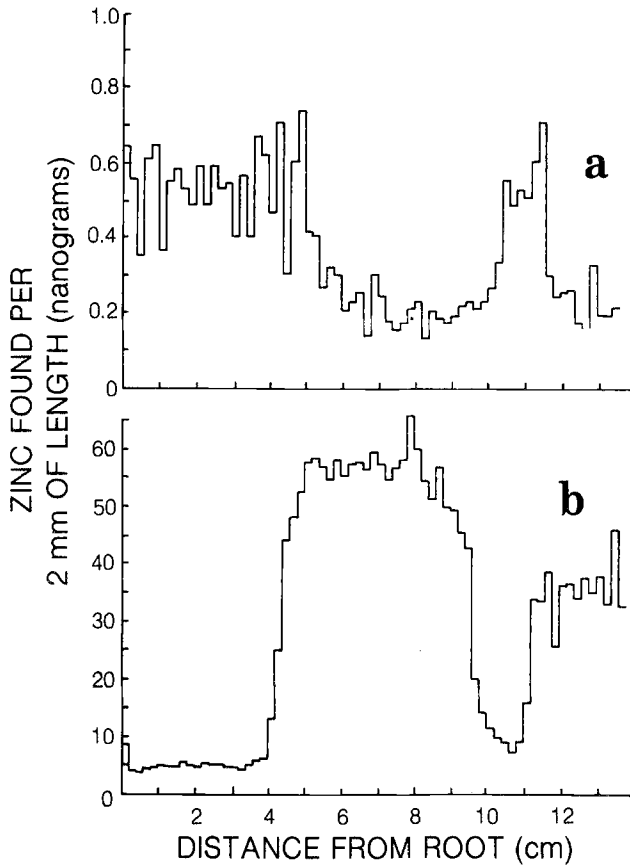


FIG. 9—Zinc content of two hairs from Subject J. Hair showed bleached regions from 5 to 10 cm and from 12 cm onwards: (a) indigenous zinc content of 1-mm segments and (b) zinc absorbed from radiotracer solution, with a soaking period of 1 h.

in corresponding anagen hairs, with merely a displacement of the pattern corresponding to the length of time since the telogen hair entered that phase of its growth cycle. If so, this would complicate matching a telogen hair with others from the same subject but should not inhibit it entirely.

Summary

The concentration patterns of indigenous zinc and of zinc and cobalt absorbed from radiotracer solutions have been measured via flameless AA and radioactivity assay, respectively, in individual human head hairs.

Patterns for indigenous zinc were found to be relatively flat and featureless. Patterns for absorbed zinc (like those for indigenous and absorbed copper in the same subjects) showed increasing concentrations with increasing distance from the root, plus zones of locally increased concentrations at positions different from those for zones of increased copper concentrations. In bleached hair, indigenous zinc concentrations were decreased, and absorbed zinc and cobalt concentrations were increased compared to values in normal hair.

The data for absorbed zinc and cobalt were interpreted in terms of a variable con-

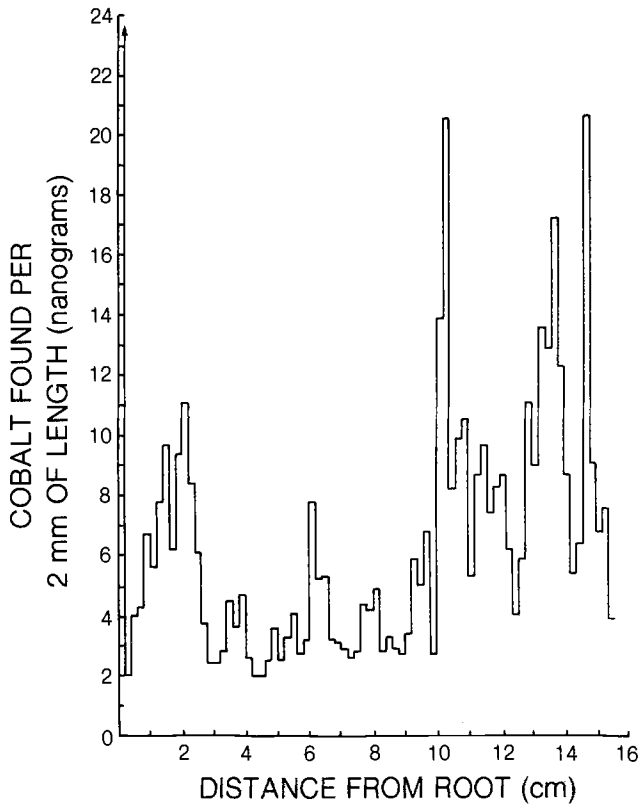


FIG. 10—Cobalt absorbed by a hair from Subject G from a radiocobalt tracer solution with a soaking time of 3½ h. Hair showed a bleached region from 9 cm onwards from the root.

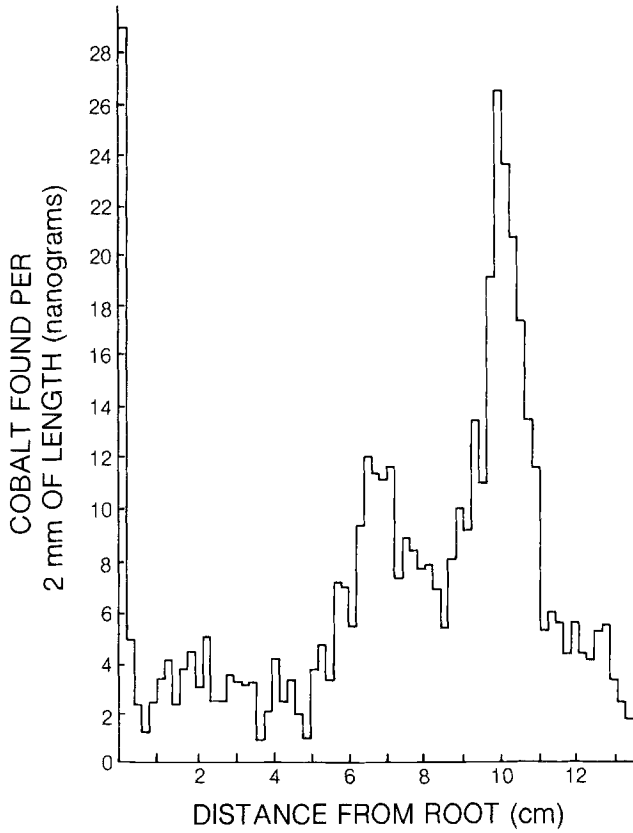


FIG. 11—Cobalt absorbed by a hair from Subject J from a radiocobalt tracer solution with a soaking time of 2½ h. Hair showed a bleached region from 5 to 11 cm from the root.

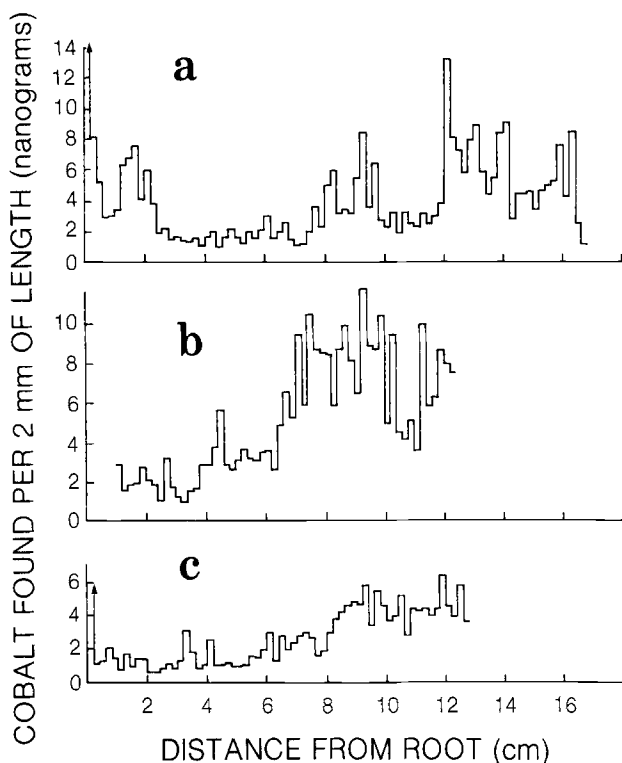


FIG. 12—Cobalt absorbed by three hairs from Subject G from a radiocobalt tracer solution with a soaking time of 91 h. Hair showed bleached regions from about 6 to 10 cm from the root and from 12 cm onwards; (a) hair unrinsed after soaking; (b) hair rinsed for 1 h; and (c) hair rinsed for 1½ h.

centration of metal-binding sites in the hair structure, coupled with an increased porosity induced by hair bleaching. The flat patterns for the indigenous zinc content were interpreted as indicating the importance of dietary zinc and incorporation via the follicle, and the unimportance of external contamination as the source of this zinc. The forensic implications of the data have been discussed.

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