J Forensic Sci, Apr. 1979, Vol. 24, No. 2

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Absorption Patterns for ^{32}P Phosphate into Single Human and Animal Hairs

Human hair has been extensively analyzed for its trace element content in various contexts. The following references are typical of work in the respective fields: toxicology [*1*], nutrition [*2*], environmental pollution [*3*], and forensic science [*4*]. The current literature on hair analysis has been reviewed by the authors [*5*].

Detailed studies by Renshaw et al [*6, 7*], Obrusnik et al [*8*], and Maes and Pate [*9-11*] have revealed notable features in the trace element concentration profiles along single hair shafts. An increase in the concentration of most trace metals in the hair of many subjects was found between the root and the distal end of the shaft. Studies with copper and zinc tracers [*9,10*] indicated that such a concentration increase may arise from an increasing capacity of the hair structure to absorb or bind metallic ions. Arsenic tracer [*11*] showed that the corresponding absorption capacity for this element decreased with distance from the root. Certain subjects were found [*9*] to have regions 10 to 20 mm long in the hair structure capable of increased copper absorption from external sources as compared with adjacent parts of the hair, and similar regions of increased zinc absorption were also found [*10*] but in different locations. Hair from certain subjects was also shown [*11*] to exhibit very sharply peaked arsenic absorption patterns.

Such concentration patterns are not characteristic only of the human species. Animal hair also actively absorbs and stores trace elements, as has been demonstrated by isotopic studies [*12-14*]. Flynn et al [*15*] attempted to determine the past trace element uptake in a wild animal by longitudinal analysis of its hair. Domestic animals have also been briefly studied [*16*].

The present experiments were intended to address the problem of anion absorption by human hair via application of ^{32}P radiotracer in the form of phosphate. Such an ion was expected to bind effectively to anion binding sites; this particular radionuclide also has specific radiation emission properties (β, but no γ radiation) that permitted determination of absorption patterns nondestructively—by a scanning technique—and hence repeatedly on the same hair shaft. This was expected to eliminate the uncertainty introduced into such studies by hair-to-hair variations in absorption characteristics.

Experimental Techniques

Phosphorus-32 radiotracer solutions were obtained from New England Nuclear Corp., Boston, Mass.; they had a reported specific activity of 10 mCi/ml and the activity was in the form of phosphate. After incorporation into a hair shaft, the radioactivity was assayed either by scanning down the length of the hair with a Si(Li) β detector collimated to a diameter of 2 mm, or by an uncollimated stationary detector positioned over individual

Received for publication 12 July 1978; accepted for publication 23 Aug. 1978.

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segments cut from the hair shaft. Data acquisition was achieved with conventional electronic equipment.

Hairs for the present experiments were acquired by plucking them from one female human subject, or by plucking or collecting them from bed sites in the case of animals; the hairs were washed by means of the previously used procedure [*8*] involving an ether-acetone-water cycle, ending with ether, and then air-drying. The human hairs were all identified as being in the anagen phase of the growth cycle via microscopic examination of the root. This was not possible for the animal hairs, which often had no root attached.

The hairs were soaked in the radiotracer solution in polyethylene vials; the activity and pH were adjusted as required, but they were usually 0.5 to 1 mCi/ml and 5.5, respectively (chosen to give satisfactory absorption intensities). After soaking, the hairs were rinsed briefly with water to remove tracer solution wetting the hair surface, air-dried, and then mounted for radioassay. As noted above, some hairs were cut into 2-mm segments by a previously reported technique [*9*] and each segment was mounted on an aluminum planchet for radioassay. Aliquots of the radiotracer solution were similarly mounted for detector standardization.

Results

The first measurement made was of the absorption of ^{32}P from solution into a single human hair over a soaking period of 17 h. The resulting radioactivity pattern is shown in Fig. 1, and the striking peaks and valleys are very reminiscent of those observed pre-

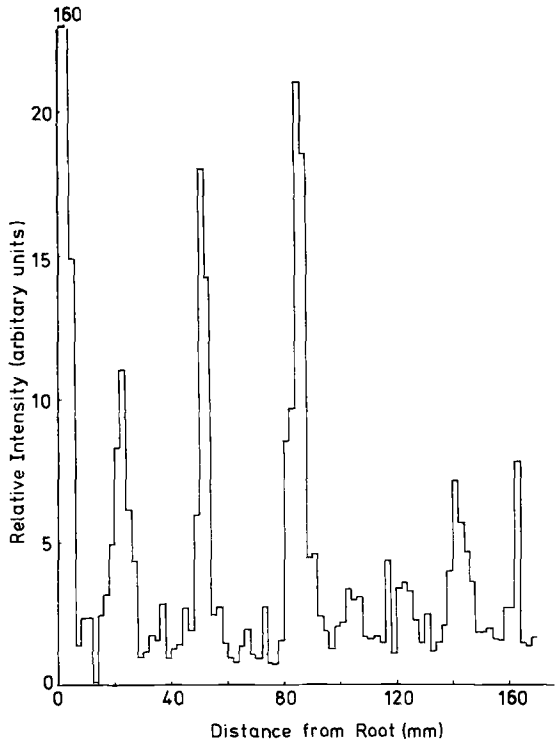

FIG. 1—*Intensity of absorbed ^{32}P activity in a single human hair as a function of distance from the root. Hair was soaked in ^{32}P solution of 0.5 mCi/ml concentration and at pH 5.5 for a total of 17 h.*

viously [*11*] in the case of arsenite absorption patterns observed for certain other subjects. Later figures in this paper show similar patterns observed with other human head hairs.

The availability of animal hairs allowed exploration of whether or not such patterns are a feature only of human hairs (and hence perhaps related to human hygienic habits or some similar species-related characteristic).

Figure 2 shows the patterns observed for two guard hairs from a grizzly bear (*Ursus horribilis*) collected from a bed site and presumed to be from a single individual, while Fig. 3 shows the patterns observed from three hairs from another such sample. Structural patterns are observed although various patterns differ in average intensity.

Figure 4 shows the pattern obtained for a polar bear (*Thalarctos maritimus*). Structural patterns, in this case, are superimposed on a curved background. Figure 5 shows the pattern found in two hairs taken from the carcass of a black bear (*U. americanus*). All the hairs show similar patterns, but two are of higher intensity than the others.

A small survey of hairs from black, grizzly, and polar bears detected no significant pattern correlations specific to species or to hairs all drawn from a single animal [*17*]. In view of the apparent similarity of behavior of human and bear hairs, these were used interchangeably in the experiments that followed.

In an attempt to understand the origin of patterns in human or animal hairs, several hypotheses were explored. Presumably the sharp peaks arise from one or both of two causes: either the hair shaft structure contains phosphate-binding sites distributed down the shaft with a highly variable density, or the exterior of the shaft (especially the cuticle) exhibits a permeability to ions that also varies sharply from place to place.

One consequence of the latter circumstances, if the ions also had a mobility along the hair shaft comparable to that in the radial direction, would be that a sharply peaked pattern generated after a short soaking period might be washed out on prolonged soaking as the ions diffused laterally.

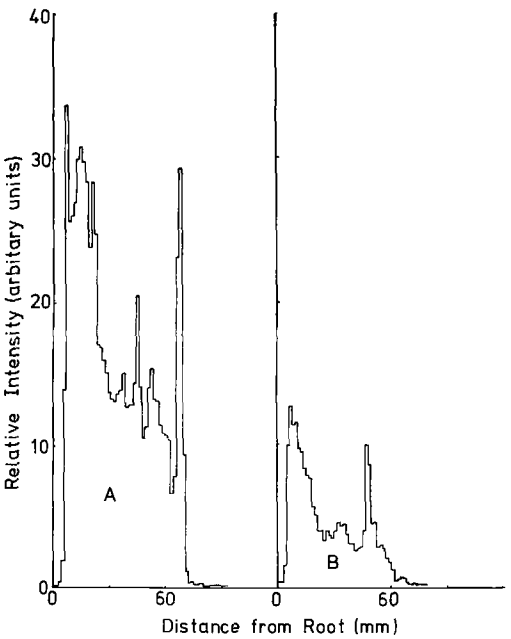

FIG. 2—*Intensity of absorbed ^{32}P activity in two single grizzly bear hairs as a function of distance from the root. Hairs were soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total of 17 h.*

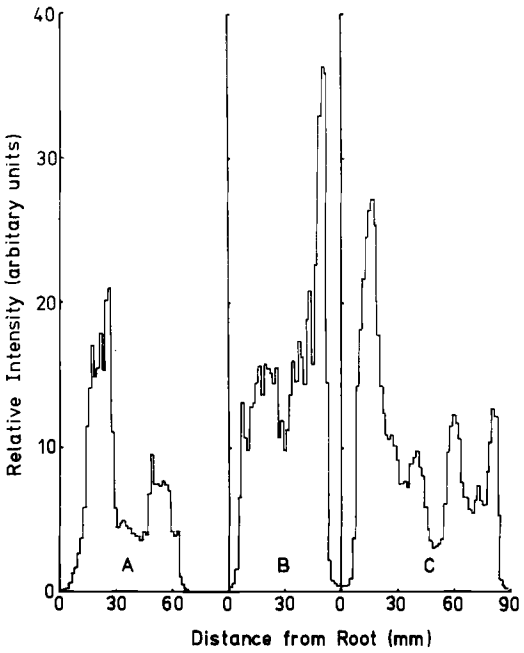

FIG. 3—*Intensity of absorbed ^{32}P activity in three single grizzly bear hairs as a function of distance from the root. Hairs were soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total of 17 h.*

The scanning nondestructive radioassay technique allowed experiments to be designed to look for such an effect. First, human hair was assayed after 19 h of uniform soaking in ^{32}P solution and then was further soaked to a total of 37 h and assayed again. The results are shown in Fig. 6. After 19 h the pattern exhibited a monotonous intensity decrease

FIG. 4—*Intensity of absorbed ^{32}P activity in a single polar bear hair as a function of distance from the root. Hair was soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total of 17 h.*

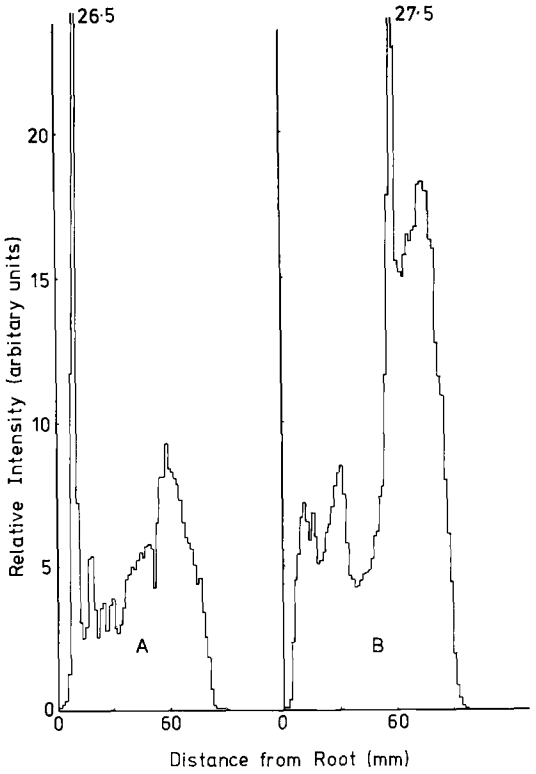

FIG. 5—*Intensity of absorbed ^{32}P activity in two single black bear hairs as a function of distance from the root. Hairs were soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total of 17 h.*

with increasing distance from the root, just as did some hairs subjected to soaking in radioarsenic solution in previous work [*11*] and in contrast to the increasing intensity observed with cationic tracers. After 37 h, however, sharp concentration peaks had appeared, superimposed on the previous pattern. This result is typical of many such observed; peaked patterns appeared and disappeared more or less abruptly in a nonreproducible way, and without obvious cause, but no evidence of pattern saturation (or filling in between peaks) was seen with soaking periods up to 48 h.

Figure 7 shows results of an experiment designed to explore shorter time periods; a single black bear hair was repeatedly soaked and assayed after total soaking times of 2, 4, and 6 h. Minor relative peak intensity changes are seen, but the structural pattern was evidently well developed in this case within 2 h of the beginning of the experiment.

More direct evidence of migration of ions along the hair shaft was sought in additional experiments. In one, the first 2 mm, including the root, was cut off a prewashed human hair, and the next 2 or 3 mm of the shaft was then dipped into the ^{32}P tracer solution with the rest of the shaft supported vertically. After various periods of time, the hair was removed from the solution, the last 2 or 3 mm rinsed with water (not the entire shaft, to avoid possible contamination with tracer), and the pattern assayed. Figure 8 shows the results for total soaking times ranging from 1 to 95 h. Aside from the initial increase in activity absorbed from solution by the immersed segment, no other changes are noticeable, and particularly there is no tendency apparent for the absorbed activity to migrate along the shaft to adjacent unimmersed regions. An essentially identical result was obtained with a single black bear hair soaked up to 182 h.

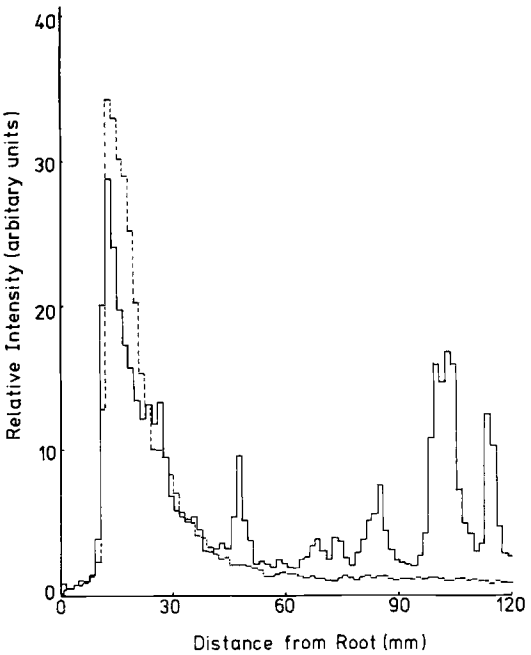

FIG. 6—*Intensity of absorbed ^{32}P activity in a single human hair as a function of distance from the root. Hair was soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total time of 19 h* (broken curve) *and 37 h* (solid curve).

There appeared to be nothing special about the particular hair chosen for this experiment; the bear hair developed a moderately structured pattern when subsequently soaked over its entire length (Fig. 9). Also, there appears to be nothing special about the root end of the shaft, nor the fact that the interior of the shaft structure was exposed in the experiment of Fig. 8. Figure 10 shows the results of a similar experiment in which a central 10- to 20-mm segment was soaked. No evidence is seen of longitudinal migration of the activity in this experiment either, although the intensity of absorbed activity in the central region initially increased and then decreased again. Apparently small changes in the experimental conditions can cause activity to move into or out of the hair structure. For example, although care was taken to avoid them, changes might conceivably occur in the pH of the solution from which absorption occurred, perhaps in small regions close to the hair shaft surface. Experiments were therefore performed to investigate the effect of pH changes on pattern development.

In Fig. 11 are shown the results of an experiment in which a single black bear hair was subjected to five 18-h cycles of soaking followed by assay, in which pH was progressively decreased from 9 to 3. A decreasing pH sequence was chosen following results in the literature [*18*] that indicated that anion absorption capacity in hair would correspondingly increase, thus facilitating measurement of absorption capacity changes. The results indicate that absorption of ^{32}P by the hair shaft generally increases as the pH is decreased from an initial value of 9 and suggest that some peaks in the concentration pattern first appear at pH values below 7, becoming perhaps stronger as the pH is reduced further. The pattern observed after soaking at pH 3 is, however, of a reduced intensity and falls out of the present general trend with pH and that reported in the literature.

After the ^{32}P radioactivity in the hair had decayed essentially completely, this same hair was subjected to soaking in a solution of ^{65}Zn at a pH of 5.5 and then cut into 2-mm

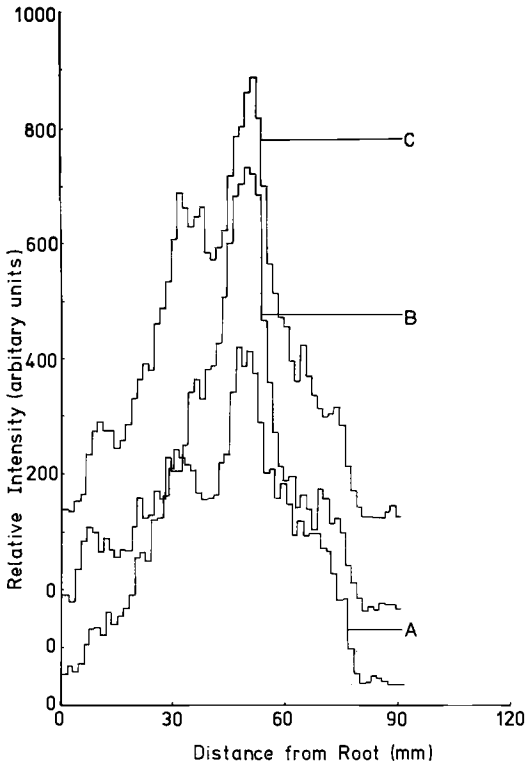

FIG. 7—*Intensity of absorbed ^{32}P activity in a single black bear hair as a function of distance from the root. Hair was soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total time of 2 h* (Curve A), *4 h* (Curve B), *and 6 h* (Curve C).

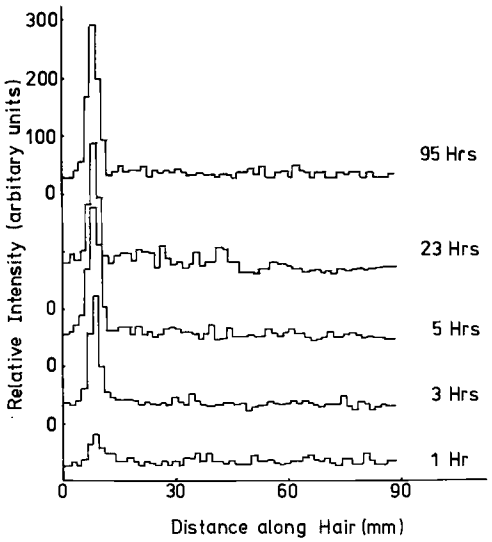

FIG. 8—*Intensity of absorbed ^{32}P activity in a single human hair as a function of distance along the hair. The last 2 mm of the hair was soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total time ranging from 1 to 95 h. For other details, see text.*

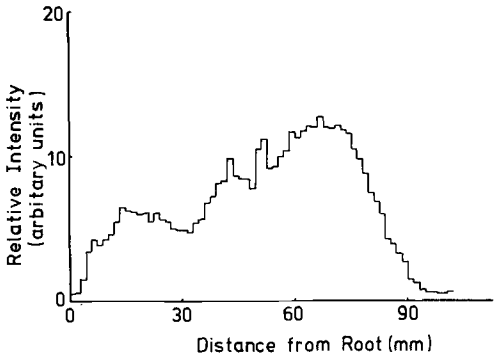

FIG. 9—*Intensity of absorbed ^{32}P activity in a single black bear hair as a function of distance along the hair. The hair, previously used in the experiment of Fig. 8, was now soaked over its entire length in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total time of 18 h.*

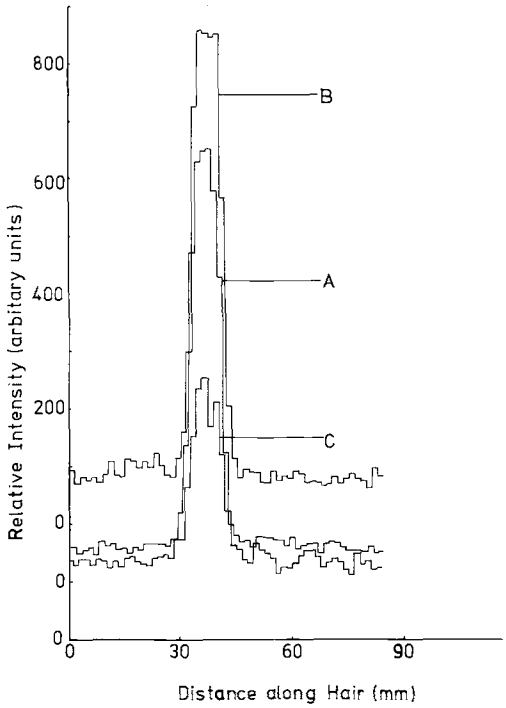

FIG. 10—*Intensity of absorbed ^{32}P activity in a single black bear hair as a function of distance along the hair. The central 10 to 20 mm of the hair was soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5.5 for a total time of 5 h* (Curve A), *7 h* (Curve B), *and 25 h* (Curve C).

segments, which were then individually assayed. Figure 12 shows the result, with the zinc activity pattern compared with that measured earlier for ^{32}P in the same hair at about the same pH. It is seen that they are complementary, with the regions of high zinc absorption being those of low phosphate absorption and vice versa.

The results of the pH variation experiments, and of other similar ones with human hairs that gave a somewhat less clear-cut outcome, posed some questions as to whether phosphate-binding sites were activated or deactivated by prior exposure of the shaft to solutions of pH higher or lower than those from which the ^{32}P was subsequently absorbed. This

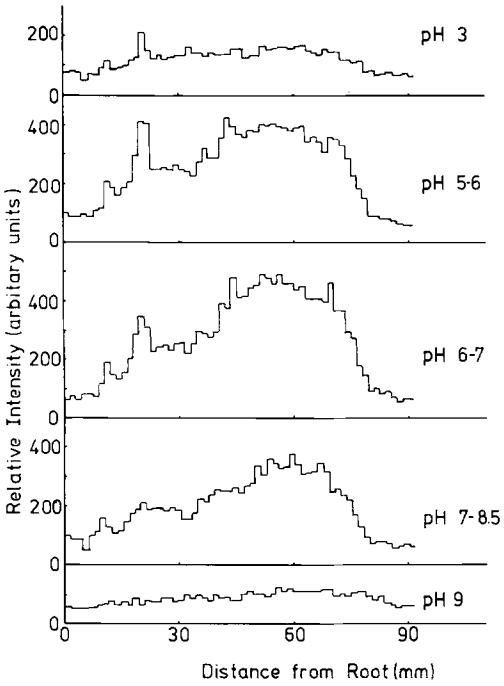

FIG. 11—*Intensity of absorbed ^{32}P activity in a single black bear hair as a function of distance from the root. Hair was soaked in ^{32}P solution at 0.5 mCi/ml concentration for five 18-h cycles in which the pH was progressively decreased from 9 to 3.*

was checked with a single human hair which, in separate experiments, was conditioned by soaking at pH 10 and then at pH 1, in each case prior to soaking in ^{32}P solution at pH 8. No significant difference in the two activity patterns was evident. In contrast to this was the result obtained using three human hairs, one (C) being given a brief wash with an acidic solution and then a water wash, another (B) an alkaline wash followed by water, prior to a soaking in ^{32}P solution, and the third (A) only the usual presoaking wash treatment. As can be seen in Fig. 13 the acidic washed hair exhibits a peaked pattern, whereas the alkaline washed hair is similar to the control hair. The implications of these results are not clear but suggest that any pretreatment of the hair should be carefully controlled. In a separate experiment it was demonstrated that no difference in absorption pattern was evident if a hair was air-dried prior to being soaked in tracer solution or if it was saturated with water.

Conclusion

The present work, together with previously published results, indicate that both human and animal hair shafts have the capacity to absorb and bind trace elements from the hairs' environment, this capacity generally (but not always) increasing with distance from the root for cations and decreasing for anions. Superimposed on this general trend, however, there is often a variation of absorption capacity that can lead to peaks in the concentration pattern of the absorbed material; the present work has shown that such peaks can be of dramatic intensity in the case of phosphate absorption, as previously shown for arsenite [*11*]. However, all the factors that lead to formation of such concentration peaks have evidently not been identified or controlled; in experiments with single hairs, peaked patterns occasionally came and went with no obvious change in the experimental conditions being observed.

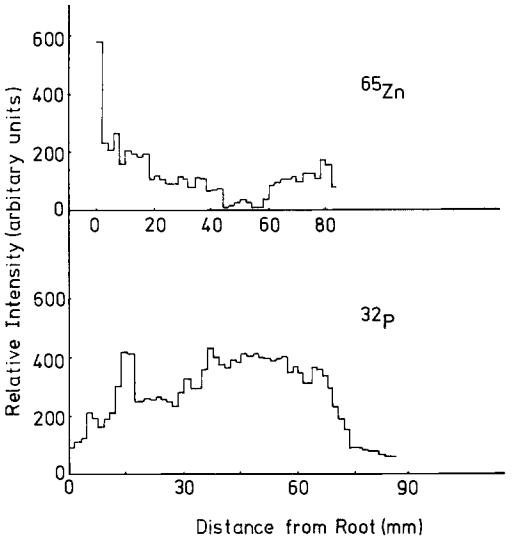

FIG. 12—(top) *Intensity of ^{65}Zn activity absorbed into a single black bear hair as a function of distance from the root (after soaking in ^{65}Zn solution at 0.2 mCi/ml concentration and at pH 5.5 for 18 h).* (bottom) *Intensity of absorbed ^{32}P activity in the same hair as a function of distance from the root (curve for pH 6 to 7 from Fig. 11).*

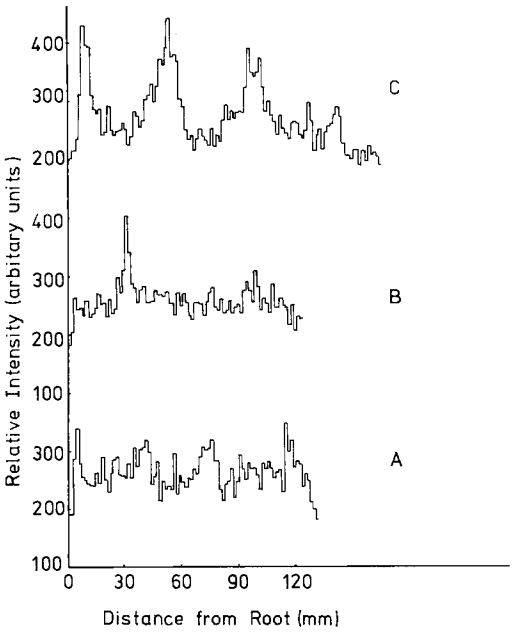

FIG. 13—*Intensity of absorbed ^{32}P activity in three single human hairs as a function of distance from the root. Hairs were soaked in ^{32}P solution at 0.5 mCi/ml concentration and at pH 5 for a total time of 18 h after various pretreatments*: (A) *normal washing procedure*, (B) *acidic wash, and* (C) *alkaline wash.*

Among the factors apparently not responsible for peak formation were the effects of human hygiene (since animal hair behaved in the same way as human) and migration of phosphate ions along the hair shaft. Decreasing the pH of the solution to which the hair was exposed was, however, found to favor peak formation. This supports the presence of anion-binding sites within the shaft structure, evidently (for some subjects) exhibiting a highly nonuniform spatial distribution, which may be ionized—and hence available for anion binding—at pH values below 7. Since sweat in contact with hair has a pH of 5.5 or so, there is a possibility of such peaked patterns existing for trace anionic species present naturally in hair, but this has yet to be confirmed by experimental observation.

Summary

Absorption of phosphate into single human and animal hair shafts has been studied by means of ^{32}P radiotracer techniques. Nondestructive radioassay permitted repetitive experiments on the same hair specimen.

Phosphate concentration patterns were shown to be complementary to those for zinc absorption and sometimes to exhibit prominent concentration peaks extending over regions of the shaft 10 to 20 mm in length. Appearance of such peaks was shown to be favored by pH decreasing below 7 but not by migration of phosphate along the hair shaft, which was unimportant.

Acknowledgments

The authors are grateful to B. L. Smith and R. M. F. S. Sadleir for supply of the bear hair specimens. Support of this work by the National Research Council of Canada, under Grant No. A2510, is gratefully acknowledged.

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