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TRACE ELEMENT CONCENTRATION PATTERNS IN HUMAN HAIRS

by

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Maitrise es sciences, University of Paris, 1969
Doctorat 3° cycle, University of Paris, 1971

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Thesis Title: "Trace Element Concentration Patterns in Human Hairs"

Abstract:

Measurements of the variation of copper and zinc concentrations along single human head hairs have been made by flameless atomic absorption spectrophotometry. The concentration patterns as a function of distance from the hair root are similar for hairs from the same head but substantially different for hairs from the several different heads studied. Experiments on hairs soaked in solutions containing radioactive copper have shown strong similarities between the concentration pattern of natural and added copper along the hair shaft. These data, plus those from hair measurements after increased copper ingestion, suggest that the observed natural concentration patterns may arise entirely from copper from external sources.

Measurements of the amount of radioactive zinc, cobalt and arsenic, absorbed separately in single hairs, provide evidence that each of these metals is preferentially bonded to specific regions of the hair shaft, which recede from the scalp as the hair grows, and which are characteristic of the hair of a given individual.

Finally experiments have been conducted in which the elements mercury, antimony, silver, zinc, and cobalt were absorbed simultaneously on the same hair. The total quantity absorbed and the concentration pattern were observed to be different from element to element and from subject to subject (for a given element). The chemical significance of this information is discussed.

A Corinne et Valérie

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1) INTRODUCTION

1.1) General and Physiological Considerations

Trace element analysis has become increasingly important in recent years, due to the concern over trace element content in food and in the environment.

Almost all of the studies undertaken so far in this area have been mostly concerned with the effect of trace elements on man.

In the medical area, there is a greater awareness of the role of trace elements in nutrition, in normal metabolic processes, and especially in causing, directly or indirectly, abnormal physiological effects.

Analysis of hairs for their trace element content has been of special interest since 29 trace elements have been found in human hair (Gui. 66b), and because the levels of most of these trace elements are much higher in hair than in other commonly analyzed tissues or fluids such as blood and urine.

Because of the slow rate of growth of head hair (about 1 cm per month), and because many of the metals excreted by the body through perspiration or appearing in the blood become bound to some extent to hair proteins, hair may have the potential of concentrating many such

elements and perhaps revealing a profile of environmental exposure.

In addition, hair is one of the most durable parts of the body, so that such profiles may also last for some time after their generation. Historical or archeological applications of trace element analysis in hairs have consequently been described (For. 61, For. 64) as providing useful environmental information from times past (Gor. 73, Wyt. 72).

Before we review the fairly important literature on trace element analysis in hair, it is relevant to discuss the structure of the hair shaft, whose inhomogeneity and complexity may explain some of the interesting features of the behaviour of trace elements in hair.

1.2) Structure of the Hair Shaft

Hairs are made primarily of keratins, which are a group of proteins produced in the epithelial cells of higher vertebrates. This group of proteins form also the main bulk of the horny layer of the epidermis and of other epidermal appendages such as nails, scales and feathers.

The hair shaft structure consists of three distinct regions: the cuticle, the cortex, and the medulla.

1.2.1) The Cuticle

The cuticle forms the outermost part of the hair, and as such, regulates the absorption and adsorption of trace elements in the keratin fiber. This region has a rather inhomogeneous structure since it is composed of a keratinous part (the exocuticle) surrounding a zone consisting of cellular debris (the endocuticle). The surface of the cuticle is covered by a layer, 30 Å thick (Kin. 68), which is resistant to alkalis, acids, and oxidizing agents, and consequently provides a strong mechanical and chemical protection against the environment. Disruption of the epicuticle will accelerate the absorption of trace elements into the hair structure.

1.2.2) The Cortex

The cortex, which represents the bulk of the hair fiber, consists of an aggregate of filaments (called microfibrils) consisting of low-sulfur proteins, embedded in a homogeneous matrix which is essentially composed of high-sulfur proteins (with amino acids such as cysteine). Although the diameter of these microfibrils has been found to be substantially constant along the hair fiber, the distance between adjacent microfibrils varies considerably, being a function of the content of high sulfur proteins in the cuticle. It has been shown that the matrix content increases in wool from sheep maintained on a cysteine-rich diet (Gil. 64). This suggests that the proportion of

matrix existing in the structure is a function of the diet, fluctuations in which will create an inhomogeneity along the hair fiber.

1.2.3) The Medulla

The central portion of mammalian hairs is composed of vacuolated medullary cells whose cystine content is very low. This region of the hair shaft has a considerable influence on the mechanical properties of the hair fiber, since the fibrous content of the medullary cells is concentrated peripherally against the cell wall producing a kind of frame which increases the stiffness of the hair fiber (Mer. 61).

Although it is not actually known whether trace elements can penetrate as deep as in the medulla, it seems that the very dense structure provided by the network of microfibrils embedded in the matrix, which together form the bulk of the cortex, should bind most of the trace elements during their migration towards the central core of the hair shaft.

Furthermore, the presence in the cortex of the cell membrane complex, which is formed from the plasma membranes of the developing cells during the keratinization and forms an adhesive layer between the content of adjacent cells, will tend to hinder the migration of trace elements into the keratin.

1.3) Hair Growth Cycle

The cyclic growth of animal hair can be divided into a series of steps or phases:

The anagen phase - Protein production and cell division occurs vigorously in the follicle, keratinization occurs in the canal leading to the surface, and the external hair grows in length, as newly produced hair shaft emerges from the scalp. This phase is approximately 1000 days long for human head hairs.

The catagen phase - Growth slows to a stop. The root shrinks and becomes club- or bulb-shaped.

The telogen phase - (Approximately 100 days long for human head hair). The follicle is inactive; the external hair remains the same length, attached to the scalp only by virtue of the fact that the bulb-shaped root is larger in diameter than the opening in the scalp surface. The hair will eventually be lost from the head, either by mechanical removal during the telogen phase, or when a new hair is produced as the follicle resumes activity in the next anagen phase (although sometimes the new hair may coexist with the old hair for periods ranging from 1 to 8 weeks).

Since man is, together with the guinea pig and cat, one of the few species with cyclic follicular activity randomly distributed among the hair follicles, two hairs plucked from the same head may well be in different phases of the growth cycle, which could result in trace element concentration pattern features, laid down at the same time, to appear at different distances from the root. If the phase must then be known, it is important for each individual hair to be plucked with the root attached, since distinction between anagen and telogen hairs is best achieved according to the healthy or atrophied condition of the root (see Fig. 1). In most cases, the distinction is easy to make, since anagen hairs have heavily pigmented roots, which are usually surrounded by a layer of white root sheath cells. Telogen hairs, in contrast, have club-shaped roots, no pigmentation, and very often no white sheath.

In a few cases, however, phase determination is difficult because of a natural atrophied appearance of the root even when it is active. The only point of reference in this case will be the pigmentation of the root, and therefore the phase determination is uncertain.

Since it is impossible to determine with precision the length of time a given hair follicle entered into the telogen phase, only anagen hairs should be used if a correlation between, say, exposure time and the location of a specific feature incorporated into the hair shaft has to be attempted, although "a broad agreement between

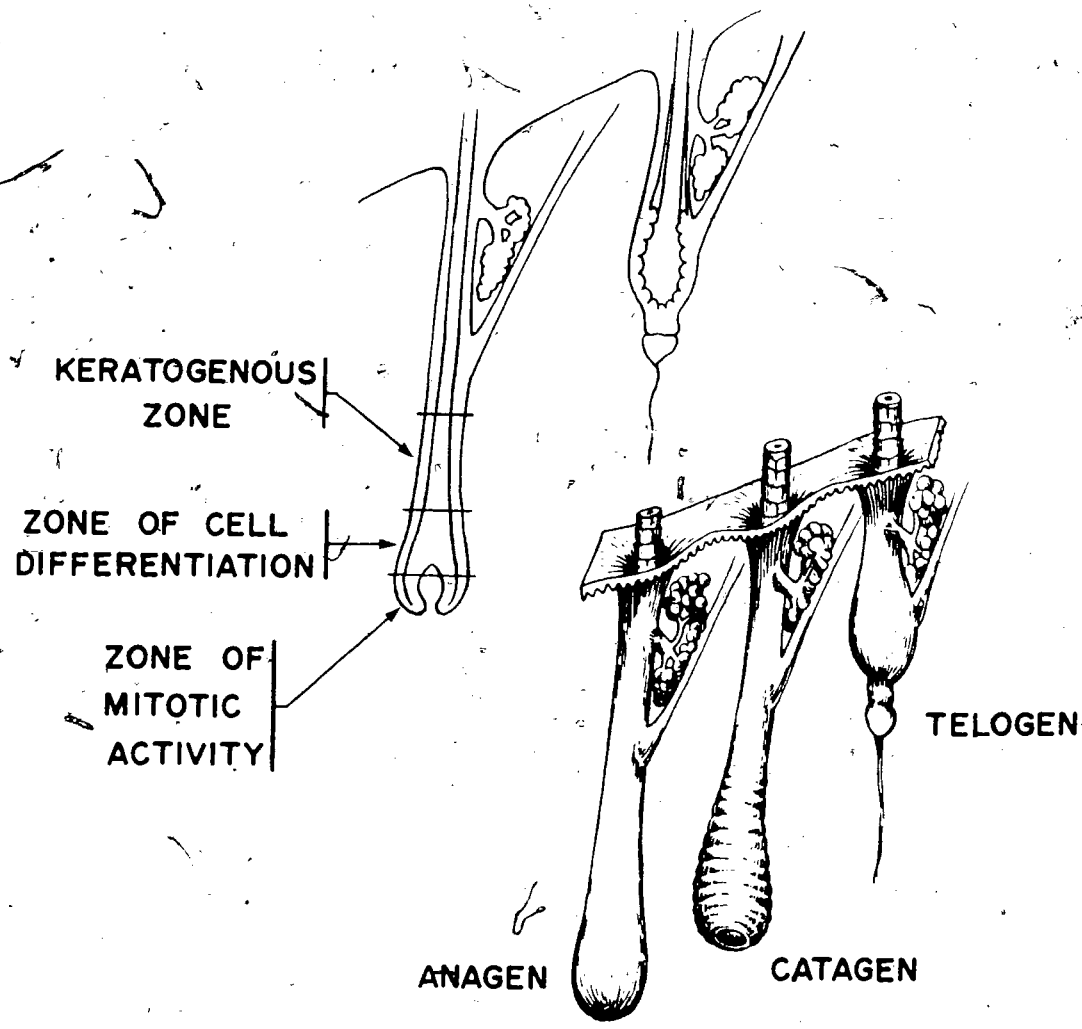


FIGURE 1 Diagram of a growing and a quiescent follicle. (After: the Biology of Hair Growth, Montagna W., Ellis R.A., Ed. Academic Press Inc., p.48 (1958)

sectional profiles and the date of application of a lotion containing Hg and Se" (sic) has been found with hairs in the telogen phase (Gan. 72).

1.4) Hair Growth Rate

The other important parameter which can affect the location of hair pattern features is a variation in the rate of hair growth. Measurement of this quantity for every hair analyzed is, however, not practical, and one must use the average values found in the literature. Average daily growth of scalp hairs has been measured as 0.35 mm (Mye. 51), and this value is presumably adequate, if only a rough estimate of a time span is wanted. For more precise values it is necessary to take into account the variation of growth rate from hair to hair on a single head, plus the effects of such factors as hormonal fluctuations, seasonal and diurnal variations, effect of age, and the influence of possible physical treatments (such as mechanical stimulation or hair growth lotion) (Fle. 54).

It seems possible, however, that changes in the hair rate of growth may be fairly uniform over a whole scalp, since they are governed by common physiological factors. To the extent to which this is true, it should be possible to correlate the concentration patterns in several anagen hairs plucked from a single head one with another, and with the time at which an important modification of the subject's health or diet may have occurred.

1.5) Trace Element Metabolism

1.5.1) Role of Trace Elements in Living Organisms

The analysis of human hair for its trace element content has not only attracted interest in the field of forensic science, but is now used as a monitor of excessive or inadequate ingestion of various metals (Str. 66; Kle. 70a), as a severe deficiency of an element could be associated with low concentrations in hairs (Rei. 66)(Pra. 66).

It has been proved that not all trace elements have an equal influence on the "well being" of animals and plants. Some elements, including some occurring in low concentrations only, are essential for their life and growth, whereas more or less complete absence of other (non-essential) elements will have no observable injurious effect.

Essential trace elements are believed to exercise a mostly catalytic function in living organisms, either in their own right, or as the prosthetic group or activator of a given enzyme, such as copper in the case of tyrosinase. Thus, only minute variations in their concentrations may considerably affect such important functions as respiration (copper) or normal growth of plants (molybdenum).

Furthermore, interactions between various trace elements can

modify the amount of mineral nutrients essential for the full development of the organism. It is, for example, well known that calcium intakes reduce the availability of dietary zinc (Obe. 66), resulting in the possibility of zinc deficiency for subjects subjected to a diet rich in calcium.

It is therefore difficult, not only to fix the minimum amount of mineral required for a balanced nutrition, but even to relate a malnutrition symptom with a dietary deficiency in a given metal.

Because of the fairly complicated and imperfectly understood way essential trace elements are excreted by the body, (and perhaps incorporated into hair), the use of trace element content of hair as an indicator of mineral malnutrition relies only on experimental data which indicate that a correlation may exist between these two factors, and such use may therefore be subject to limitations.

It seems, futhermore, that different conclusions exist as to whether a correlation can be found between the levels in plasma and in hair of a metal such as zinc (Str. 66), as this has been recently refuted (Kle. 70a).

A definite correlation does apparently exist between trace element concentration in hairs and such factors as age (Pet. 71, Sch. 69), sex (Pet. 71, Kle. 70a), hair color (Sch. 69), and even

seasons of the year (Str. 66). In particular, the consistently high concentration of essential elements such as Mg, Zn, Cu, and Mn found in the hairs of newborn and young children, which declines during the first decade of life and remains constant thereafter (Sch. 69), can be contrasted with the concentration of non-essential elements such as cadmium and lead, which accumulate in human tissues with increasing age.

1.5.2) Role of Trace Elements in the Formation of Hair

It has long been recognized that diet affects not only the general growth and development of an organism, but in particular the condition of the coat of an animal. For human beings, however, a very poor diet is needed to cause actual hair loss, probably because a smaller fraction of the total available protein is diverted to hair formation.

The rate of fiber production is definitively influenced by the dietary content of proteins, amino acids such as cysteine or methionine, carbohydrate, and fat (Ryd. 58). Similarly, specific deficiencies in vitamins (Vitamin A, riboflavin, Vitamin E) and minerals such as copper, zinc, iron, cobalt, and phosphates (Ryd. 58) impair the production of wool in sheep and, to a lesser extent, the rate of hair growth in man.

The nutrition of the root is achieved through a network of blood vessels whose density is high around the keratogenous zone and the lower part of the follicle, where the mitotic activity takes place (see Fig. 1).

Since the injection of cysteine labelled with ^{35}S resulted in appreciable radioactivity appearing in the keratogenous zone of active follicles (Ber. 54), it seems reasonable to assume that the necessary nutrients for hair growth are supplied to the root through the network of blood vessels.

Presumably, of those essential for keratin formation, some quantity of the trace elements which are found in the hair shaft are incorporated by this process.

Copper, especially, plays an important role during the keratinization process, by catalyzing the oxidative closure of the sulfhydryl group of cysteine into the disulphide bond of cystine (Rot. 65). This S-S bond is in part responsible (together with the hydrogen bond and the salt bond) for the cohesion of the keratin structure; indeed, copper deficiency results in brittle hairs. Copper also plays an important role in the pigmentation of hair. Melanin, which is the pigment responsible for the black-brown color of hair, is formed by the catalytic action of tyrosinase (which is a cuproprotein) on tyrosin (Fit. 58), and the activity of this enzyme is related to

the presence of copper in the follicle (Fit. 58).

However, it is not possible to conclude that the presence of Cu in the hair shaft is entirely due to its incorporation in the growing region of the hair bulb, since copper is common in the environment and external contamination is expected to be an additional source.

Similarly, zinc (Fol. 66) and iodine (Ker. 64) are suspected to be essential for the growth and the pigmentation of hair, since these elements have been shown to be present in the follicle (Mon. 58), and because loss of pigmentation and/or impaired growth is a consequence of a deficiency in either of these elements.

1.5.3) Sweat Secretion and Sebaceous Excretion

Whether trace elements are incorporated into the keratin structure through hair growth or through external deposition of foreign material on the hair shaft, it seems relevant to examine briefly the mechanisms through which the body excretes significant quantities of liquids, which could be a possible vehicle for the penetration of external trace elements into the hair shaft.

1.5.3.1) Sweat Secretion

Two different glands contribute to the production of sweat: the

eccrine and apocrine glands.

The eccrine sweat glands are distributed all over the surface of the body in man, and produce an aqueous sweat responsible for heat regulation. Furthermore, even at temperatures below the critical temperature of sweating, the sweat glands periodically secrete microscopically visible sweat droplets, which rapidly evaporate from the skin surface (Rot. 65), a normal process of water loss.

Eccrine sweating is not only a response to temperature increase, but is a consequence of nervous stimuli (emotional stress and sensory stimulation) (Mon. 62). Thermogenic sweating is greater on the head and the trunk than anywhere else on the body, which is relevant for the interpretation of results such as the distribution of trace elements in hair shortly after ingestion (Lim. 66).

The chemical composition of sweat depends to some extent on the material available in the blood stream, and as such, sodium, calcium, magnesium, sulfur, phosphorus, and especially iron, have been consistently found in eccrine sweat (Mon. 58).

It seems, then, that eccrine sweat can be considered as an excretory pathway (although not the most important) for some trace elements which are in excessive concentration in the organism.

The apocrine glands do not occur over the whole body surface in man, but derive from the follicular epithelium and open into the follicular canal. The secretion of these glands is not produced by thermal stimulation, but rather by mechanical stimuli, and consists of a small amount of viscous liquid appearing at the end of the follicular canal.

Apocrine sweat contains iron in fairly important quantities (6-10mg Fe/cc (Ada. 50)) and is one of the main excretory pathways for this metal.

Although eccrine and apocrine glands have distinct canals of secretion, the proximity of these two sweat glands causes their secretions to become mixed.

1.5.3.2) Sebaceous Excretion

Like apocrine glands, sebaceous glands are associated with hair follicles on the skin of the human body. However, the excretion of fat from the sebaceous glands occurs by physiological disintegration of the gland cells, rather than as a secretion product like eccrine sweat. The excretion of sebum is regulated by the following important factors:

1) The amount of fatty material already present on the

skin determines the quantity of sebum excreted, which increases as the fatty film is removed from the skin (Mon. 58).

2) High temperature increases the amount of sebum excreted, since above 30 ^{o}C it becomes so fluid that no occlusive layer is formed. Furthermore, sweat secretion emulsifies the sebum and facilitates its spread (Jon. 51).

3) Forced feeding of animals with fats results in excretion of increased amounts of sebum, and the nutritional fat constituents are excreted unchanged.

Considerable biological significance is attributed to this lipid material as it is supposed to protect the skin and hair against the vicissitudes of the atmosphere and to slow down the absorption of foreign substances from the outside. Sebum consists of fatty acids, alcohols such as cholesterol and glycerol, hydrocarbons such as squalene, and vitamins A, E, and D.

It seems that one action of sebum is to retard the deposition of trace elements on the hair shaft, since it delays the wetting of the keratin, and as such postpones the penetration of water and aqueous solutions. However, salts of lead, tin, copper, arsenic, bismuth, antimony and mercury tend to form compounds with, or dissolve in, the fatty acids of the sebum, and as such penetrate the intact skin

through hair follicles and sebaceous glands (Mon. 58). It is reasonable to assume that these elements can be absorbed into the hair fiber in the follicular cavity itself, where the keratin is not completely hardened and hence is more susceptible to incorporate these trace elements into its structure.

Although the precise compositions and roles of both sweat and sebum are not perfectly known, they seem to play an important role in the incorporation of trace elements into the keratin structure, since they are almost continuously in contact with the hair surface.

It is clear that many different factors have a direct bearing on the presence of trace elements in hair, such as structure of the keratin and availability of the elements in the follicle and on the hair surface, together with the "living habits" of the subjects from whom the hair samples are taken.

It seems interesting now to review the extensive literature which deals with the analysis of trace elements in hair, and to see which particular features will result from the specific structure and composition of the hair shaft we previously described.

1.6) Forensic Applications

1.6.1) Large-scale Surveys

One of the most controversial applications of hair analysis for trace element content is in forensic science. Identification of individuals has been claimed to be possible through hair trace element content (measured as an average value for the sample size employed which has usually been a hair bundle of several milligrams in man). This content has been postulated to be specific for a given individual, deriving from a characteristic diet, environment, and metabolic chemistry. The problem of identifying individuals through analysis of their hair is far from being resolved however, although the determination of trace element content of human head hairs via the technique of Neutron Activation Analysis has been applied widely in this connection (Jer. 67).

Large scale surveys of the trace element concentration distributions in the hair from various populations have been conducted by Perkons and Jervis (Per. 65, Per. 66), Coleman (Col. 67), and Bate and Dyer (Bat. 64). Such studies were undertaken to demonstrate the feasibility of identification by trace element analysis of hairs, and measured the variation of trace element concentration for a whole

population. The variation of trace element concentration over a single head was also studied (Col. 67). These studies made clear that important variations of concentrations occur for whole populations (Col. 67), resulting presumably in part from variation in conditions of exposure to trace elements from place to place and from year to year. They furthermore demonstrated that in the case where several bundles of hairs taken from a single head are analyzed, the variation from bundle to bundle of concentrations of trace elements was significant (Col. 67), but still small enough (Jer. 61, Per. 62) to allow an identification by matching the trace element concentration of two hair samples.

Gordus (Gor. 73) showed recently that uniformity of diet and living habits of the population from which the samples were taken resulted in a reduction in the metal content variance, in comparison with the results obtained for a population exposed to a heterogeneous diet and environment (Per. 65, Per. 66). Whatever the degree of similarity exhibited by two sets of hair samples, it is not possible to ascertain that they have a common origin unless it has been possible to determine the number of people in a population at large whose hairs would exhibit trace element concentrations similar to those for the samples being compared, relative to the size of the whole population.

Parker (Par. 66, Par. 67) has described an attractive statistical method which not only establishes whether or not common features exist between two sets of samples but determines the proportion of the whole population from which specimens will be indistinguishable from either set of samples.

This method uses the results of the large scale surveys conducted by Coleman (Col. 67) and Perkons and Jervis (Per. 66), and becomes more selective with the number of trace elements whose concentrations are compared.

The major limitation of this statistical treatment lies in the fact that it can only be applied to the comparison of data such as mean concentration values over bundles of hairs, and not to comparisons of features like the patterns of distribution of a given metal along the hair shaft. For this kind of attribute, only the linear correlation method can be applied, and this point will be discussed later in Appendix A.

1.6.2) Single Hair Analysis

Much of the above survey work was conducted with samples consisting of hair bundles; in forensic case work however, samples frequently consist of individual hairs, and consequently, among the important questions to be studied, was evidently the variation of

trace element concentration along single hairs over a single head.

Coleman et al. (Col. 67) proved that in this case, the variation of trace element concentration was comparable with the variation for a population at large, although the hair to hair differences in concentration were reduced by choosing a washing procedure which reduced external contamination. Furthermore, the variation in trace element concentration over a seven-month period was found to be bigger than the variation for samples taken simultaneously on a single head (Col. 67). Although negative, those results emphasized the need for further investigation of the intrinsic variation of metal concentration over a single head.

Recent studies (Obr. 72) of samples consisting of 10-cm segments of single hairs have shown that the variation of the concentration of such elements as Cr, Se, Fe, Sb, and Ag can be substantial among hairs taken from immediately adjacent sites on the scalp, and all identified as being in the anagen or growing phase of the hair growth cycle (to eliminate one possible perturbing factor).

In the same study, an increase in the concentration of most trace elements was found between the hair root and the opposite or distal end of the hair, by as much as a factor of 20. Thus, if two single hair samples are to be compared under the circumstances where the distance from the hair root is not determinable, and the phase of the

hair growth cycle in each case is not known, matching by trace element content averaged over a whole hair, or even over 10-cm segments, appears to be of dubious validity (Obr. 72).

1.6.3) Single Hair Profiles

Instead, therefore, of employing average concentration values for characterization of hair, an alternative, such as the measurement of the pattern of concentration variations along the hair shaft, is needed.

The recent development of flameless Atomic Absorption Spectrometry, with the concomitant improvement in sensitivity in the determination of many trace elements, has made possible the measurement of such patterns through analysis of successive hair segments of much shorter length, and without prior dissolution (and hence with lesser risk of contamination). The technique was first applied to Cu and Pb measurements on 0.5 and 1 cm sections, respectively, of individual hairs by Renshaw et al. (Ren. 73), who demonstrated that concentrations of both lead and copper increased from root to tip, and that the standard deviation of copper and lead concentrations for the population at a given distance from the root were roughly twice those over a single head.

These results indicated that analysis of short hair sections

might be useful in discriminating between hair samples from different sources.

Similarly, Obrusnik et al. (Obr. 73) demonstrated the existence of patterns in the spatial distribution of such elements as copper, magnesium, lead, iron, silver and manganese, which could possibly be used in hair characterization, instead of the usual comparison of average concentration figures.

1.7) Effects of Hair Washing and Hair Soaking

Whatever the origin of the different trace elements which are concentrated in human head hairs, it seems likely that an appreciable quantity of them derives from external contamination (Bat. 66b) either directly or via sweat and sebaceous excretion. Thus, a question which has been discussed (Obr. 73, Bat. 65) is the desirability of removing such "external contamination" so that the analysis may reveal only the "natural" content of trace elements in the hair. It is of course also arguable that a characteristic external contamination could be used as a means of identification (Jer. 67, Obr. 72), since it might reflect the living environment of the subject (e.g. a zone of heavy pollution) or the subject's occupation (e.g. in the case of a mine or metal plant worker). In most forensic cases, however, unless the suspect is apprehended immediately after the crime, identification by means of analysis of hairs which have not been previously cleaned is

of dubious validity, since many irrelevant factors such as poor handling of the samples, may have caused the external contamination to increase.

The need for washing the hair samples prior to analysis is now generally acknowledged, although a range of opinions remains as to whether an organic solvent such as diethyl ether, acetone or ethanol (Obr. 73) should be used or rather an aqueous solution with or without a non-ionic detergent (Bat. 65) similar to the washing used for ordinary hygienic purposes.

Bate (Bat. 66a) and Bate and Dyer (Bat. 66b) in an impressive series of experiments demonstrated several important points:

1) The amount of a trace element absorbed by hairs from a solution in which it is soaked, depends both on the pH of the solution, and on the particular element considered. This was confirmed by van den Berg et al. (Ber. 67) who interpreted the results in terms of a competition for binding sites in the hair structure between metal and hydrogen ions.

2) A relatively short detergent washing removed typically some 50% of the trace element content of hairs, while a prolonged washing removed relatively little of the remainder.

This was first interpreted as perhaps providing a distinction between the loosely bound and tightly bound trace element content, and hence between that arising from external contamination and that indigenous to the hair structure proper.

However, van den Berg et al. (Ber. 67) showed that metals such as zinc and copper are absorbed from solution into hairs via a two-step process. Initially a rapid absorption takes place into the outermost parts of the hair structure, and subsequently a slower migration occurs deeper into the central regions of the hair shaft. Thus trace elements in these latter locations would be expected to be less readily released during washing, which would account for the results of Bate et al.

3) Elements such as Sb, Co, Fe, Zn, Ba, and Mn are much more readily removed from hair by complexing agents such as EDTA than are Au, Se and Ag (although conflicting data have been reported by Zeitz et al. (Zei. 69), who observed only a 25% decrease in Zn content in hairs washed for 33 hours in EDTA). This certainly suggests a difference in the nature of the binding of such elements to the hair keratin structure, which is expected if the binding sites have a specific (as opposed to general) chemical character.

Van den Berg et al. (Ber. 67) furthermore demonstrated a marked

difference in metal absorption among hairs coming from different individuals as well as among hairs all coming from one individual. This they attributed to variations in the keratin texture (and hence binding site density) from hair to hair.

The soaking and washing experiments seem to provide evidence that external contamination may be the origin of many of the elements observed in hairs. This conclusion evidently has an important consequence in a forensic context, since the concentration of many trace elements in hair could be modified by absorption of the metals into the keratin structure from external sources.

Studies prior to the present work were not capable of revealing fine detail in the pattern or distribution of trace elements absorbed along the hair shaft (Bat. 66a). Only the general increase in concentration from root to distal end was known, interpreted as having as its origin a longer time of exposure to external contamination of the distal end of a hair compared with the root end.

Lima (Lim. 66), however, in a series of experiments in which only the tip of the root end of single hairs was dipped into a sodium arsenite solution, found higher concentrations of arsenic at the distal extremities, rather than at the root ends which were dipped into the solution. This result clearly demonstrated the mobility of the arsenic taken up, which was furthermore demonstrated to take place

by capillary action on the outer surface of the hair shaft (cuticle).

Lima's data were confirmed for hairs in vivo in further experiments (Lim. 66) conducted on four subjects who ingested small doses of arsenic (Fowler's solution). Ten days after the ingestion, higher concentrations of arsenic were found at distal points (4 cm from the scalp) than at the root, even though the length of hair shaft grown in this same time would have been about 3.5 mm. In addition, higher concentrations and higher rate of arsenic uptake were exhibited by a subject with the habits of taking steam baths, and effecting daily hair washing, both of which might enhance spread of As by capillary action along the hair.

These results suggest that at least some fraction of the total amount of arsenic in hair could be incorporated into the hair via soaking in sweat, instead of simply growing into the hair structure produced in the root, as has been recently suggested (Pea. 71).

It is impossible however, to generalize this interpretation to other elements such as Zn, Cu, Ag, or Fe, since none of this kind of data has existed up to now.

1.8) Analytical Methods

Whatever interpretation is given to the presence of trace

elements in hairs, the quality of the data is dependent upon the capabilities of the method used for their analysis.

Three methods of the necessary sensitivity and selectivity are presently available to the analyst: Neutron Activation Analysis, X-Ray Fluorescence, and Atomic Absorption Spectrometry.

1.8.1) Neutron Activation Analysis

With the recent development of high resolution Ge-Li γ-ray detectors, Neutron Activation Analysis has become the most generally applied method of analysis of trace elements in hairs, as well as in other evidence material (Bry. 66, Col. 66, Gui. 66a). Advantages of this method have been described (Gui. 66b), including its sensitivity and multielement character. Some problems remain associated with this technique however:

- Availability of a nuclear reactor, with the concomitant facilities for the handling of the radioactive samples, and specialized (and expensive) γ-spectroscopy apparatus.

- Presence of large quantities of radioactive Na and Br in irradiated hairs which produce high backgrounds for the determination of other elements (or else from which radiochemical decontamination must be achieved).

- Difficulty in measurement of those elements which produce short lived radionuclides, unless the analytical laboratory is reasonably close to the nuclear reactor.

- Although in principle the method is non-destructive, long irradiations at high fluxes (which are necessary for determination of many elements in small samples) produce sample damage (For. 66) at least in the case of hair.

1.8.2) X-Ray Fluorescence Analysis

X-Ray Fluorescence, like Neutron Activation Analysis, allows the quantitative determination of several elements at a time, and the analysis of 1-cm hair samples has been reported (Zei. 69) for elements such as Zn and Ca. This method has the further advantage of being non-destructive, which facilitates the analysis of historical or archeological samples, as well as the re-analysis of the same segment of hair to confirm the precision of the method.

The major disadvantage of X-Ray Fluorescence Analysis lies in its relatively modest sensitivity (compared with NAA and AA) and hence inability to measure a wide range of elements in hair.

1.8.3) Atomic Absorption Spectrometry

Atomic Absorption Spectrometry is now a common method of

analysis, for the determination mostly of metallic elements in matrices ranging from blood serum to petroleum samples.

The basic principle of this method is simple: The element of interest in the sample is dissociated from its chemical bonds (for example, in a flame or furnace) to produce atoms in an unexcited, unionized "ground state". Isolated, unexcited atoms exhibit particularly simple absorption spectra, consisting only of transitions from the ground state to electronic excited states. If atoms in their ground states are exposed to a beam of the corresponding resonance radiation isolated by a monochromator from a suitable emission spectrum, a resonant absorption of the radiation will occur, the intensity of which is proportional to the number of ground state atoms which were initially present.

Two methods are presently available for the atomization of the samples:

- the flame atomizer
- the neated graphite furnace

In the flame atomizer, the sample is aspirated in a mixed stream of acetylene plus air (or acetylene plus nitrous oxide for the analysis of "refractory metals") and fed into the flame.

A second form of atomizer which is now emerging from the

experimental stage of development consists of an electrically heated graphite tube, into which samples may be introduced either (for liquids) via pipetting through a small hole in the center of the tube, or (for solid samples) by means of a sampling spoon inserted through one end of the furnace.

The temperature of the sample inside the graphite tube is raised in three steps: first, the furnace is heated to $\sim 95\ ^{o}C$ to dry the sample; secondly, the tube is heated to a temperature high enough to destroy the molecular species in which the atoms being analysed were initially bound (it is important, however, that the temperature inside the furnace does not at this stage exceed the temperature at which sample atomization occurs); thirdly, the furnace is heated above this temperature, and the population of atoms in the optical path of the instrument increases and the resulting absorption signal rises proportionately. During the atomization, the carbon furnace tube is flushed by argon gas in order to avoid air oxidation of the heated graphite.

Since the residence time of the atoms in the optical path is much longer in the furnace than in the flame, the furnace device will have smaller detection limits for most elements (in fact by 10^{-2} or more).

This improvement in sensitivity, combined with the advantage that solid samples can be analyzed in the furnace without prior

dissolution, makes the graphite furnace atomizer a very versatile and simple instrument to use.

Despite the major improvements which have brought Atomic Absorption Spectroscopy to the rank of the most sensitive methods of trace element analysis, interferences, which cause the analysis to be in error, are far from being completely absent. These interferences are summarized in Table I.

The presence of so many interferences shows the difficulty of elaborating general analysis procedures which apply to every element investigated, since each of them seems to require particular attention related to its own chemistry.

The determination of the quantity of element present in a sample requires the calibration of the apparatus. The ideal method consists of measuring the absorbance (the negative logarithm to the base 10 of the percent absorption signal) of several standards whose gross composition is identical to that of the sample, so that the element of interest will be subjected to the same environment in both calibration and measurement of the unknown.

The method of standard additions fulfills this condition, although it requires the availability of a homogeneous sample which must be in sufficient quantity to be divided into several aliquots.

TABLE I

Causes of interferences and their remedy in Atomic Absorption Spectrometry

CAUSES	CONSEQUENCES	CORRECTIVE ACTIONS
Ionization	reduces the proportion of ground state atoms available to absorb the resonant radiation	- decrease the temperature of atomization - add to the sample traces of easily ionized metal, e.g., Na
smoke formation during the atomization step	non-specific absorption of light	- increase the ashing temperature so the smoke formation will occur before the atomization - use a Deuterium beam (continuous spectrum) alternately chopped with the resonance radiation, and measure the absorption difference
Chemical Interferences		
1) formation of volatile compounds	the compound sublimes before the thermal dissociation of the molecule	- convert the volatile compounds to a temperature stable molecule which will decompose rather than volatilise the compound
2) formation of temperature stable compounds such as:	the compound does not readily dissociate so fewer atoms will be liberated	- use the maximum atomization temperature available
i) refractory oxides		use the graphite furnace atomizer which is constantly flushed with inert gas to exclude oxygen
ii) carbides		- avoid using the carbon furnace atomizer
iii) nitrides		- occurs only in the cases of metals such as Ti, Zr, Hf, and Ta (see 1)

This is not the case with hair, since this material has been shown to be inhomogeneous as far as trace element concentration is concerned (Obr. 73).

The precision which can be obtained in such measurements is mostly a function of the element being analyzed, and of the matrix in which it is included.

Malmstadt and Chambers (Mal. 60) mention standard deviations between 0.5% and 1% for most analyses using the flame. However, with the graphite furnace the standard deviation of the measurements is usually higher, because of the increased sensitivity, and can vary between 2% for aqueous solutions to 10%, and sometimes 25%, for samples whose concentrations are near the detection limit.

Since a good precision is a necessary but not sufficient condition for an acceptable accuracy, it is likely that the deviation between a measurement and the true value of a concentration will be greater than the above figure. In fact the accuracy may be limited by the action of other components which accompany the metal in the sample, and which may cause experimental deviation of the analytical results. If such components are absent, the accuracy will be determined by losses, contamination, and operational errors. However, the main disadvantage of this method remains that it is necessarily destructive, which hinders any repetitive analysis of a

given sample in limited quantity.

All the results obtained on the trace element composition of human head hairs before the present work were restricted to the analysis of hair bundles, resulting in a lack of information on the variation of trace element concentration in individual hairs over a single head.

Only recent work done with 1-cm and 10-cm segments of single hairs (Obr. 72, Rem. 73) revealed the existence of concentration patterns, which could be indicative of significant variation in trace element metabolism in the human body.

It seemed, therefore, relevant to start a more detailed study of such patterns, by measuring the concentration of metals such as Cu and Zn in 2-mm hair segments of single hairs. Such experiments should make possible the distinction of features such as local concentration fluctuations along the hair shaft, which could be of significant interest in forensic and clinical studies.

The results obtained during this work together with a discussion of their significance both in the field of forensic science and biochemistry will be presented after a description of the experimental technique employed.

2) EXPERIMENTAL TECHNIQUES

2.1) Hair Acquisition

Individual hairs were plucked from random locations on the heads of eleven subjects investigated in this study. As a precaution against contamination, the experimenter wore disposable plastic gloves, and the hairs were stored by encapsulation under reduced pressure (6×10^{-3} mm Hg) in pyrex tubes previously cleaned by nitric acid and distilled, deionized water.

It was shown that samples stored via this technique gave data on natural copper concentrations consistent with freedom from appreciable contamination, while hairs which were stored between sheets of filter paper gave higher and erratic values of copper concentration.

Such precautions were, however, unnecessary for hairs intended for radioisotope soaking experiments, since contamination by radionuclides is very unlikely to occur. All such samples were merely stored under atmospheric pressure in glass tubes previously cleaned by nitric acid and distilled, deionized water.

As mentioned earlier, it is important to identify the phase of the growing cycle in which the hairs were plucked prior to their analysis. In the majority of experiments, anagen hairs were to be

studied because of the potential interest of the correlation in time of patterns observed in various single hairs from a given subject head.

Microscopic examination of the hair root was done just after the sampling, in order to verify the growth cycle phase. Hairs in the anagen phase were taken to be those with a plump and pigmented root, with the white root sheath attached, in contrast to catagen and telogen hairs which have shrivelled and club-shaped roots with no root sheaths (Fle. 54).

It is important to mention, however, that at least in the case of one subject studied (subject F), phase identification was not completely positive, due to the small physical dimensions of the roots. In all other cases, hairs without a clear indication of being in the anagen or telogen phase were rejected.

2.2) Washing Techniques

As previously discussed, the washing of hair samples prior to analysis may be desirable in a forensic context, since external contamination might hide some of the features which could be used for identification purposes. Therefore, it was decided to use a well-standardized cleaning procedure, and, of those described in the literature (Bat. 65, Bat. 64, Ker. 64) one similar to that employed

earlier (Obr. 73) was selected.

Successive washings were effected in diethyl ether (Fisher anhydrous), acetone (Fisher spectroscopy grade), distilled, deionized water, acetone, ether, acetone, water, acetone, and finally ether, for time periods of five minutes per step.

In order to insure a good reproducibility of the washing procedure for different sets of samples, the tubes containing the hairs plus washing liquid were mechanically agitated in a 'Burrell' shaker. Hairs were finally dried in open atmosphere. It was found necessary to stretch the sample slightly over a piece of filter paper during this last step, in order to obtain a straight fiber, which was a necessary condition for the accurate measurement and cutting of samples of known length. The stress applied was, however, insufficient to produce an appreciable permanent change in hair length.

2.3) Cutting and Weighing of Hair Segments

The cutting and handling of hair segments which were in most cases 2 mm long was a rather delicate part of this experiment, due to the continuous risk of contamination. The cutting operation was achieved by inserting the hair in a glass capillary tube, previously

cleaned with nitric acid and distilled, deionized water, attached to a vernier equipped carriage. The cutting operation was watched through a microscope under a magnification of 50, and the length to be cut, which protruded from the end of the capillary, was measured both by the vernier and by means of a graticule in the field of view (to an estimated accuracy of 0.1 mm).

Cutting was effected by means of a stainless steel surgical scalpel mounted on the microscope stage, bearing on a quartz plate, previously cleaned by nitric acid and distilled, deionized water. This procedure and apparatus prevented contact between the hair sample and any part of the microscope stage, since the hair remained enclosed in the capillary tube until the particular segment was cut.

Because of contamination problems, the 2-mm segments were not weighed individually, and the analytical data are generally reported in terms of weight of metal per 2-mm length of hair. In addition, this avoids any problems due to weight change by water evaporation during storage.

In order to obtain an approximate value of the metal concentration by weight, however, a 1-cm segment was cut and weighed periodically along the length of the hair. It was assumed that the variation of weight along the hair shaft was proportional to the distance from the root, and that the weight of a 1-cm segment would

represent correctly the average over five 2-mm pieces. More detailed studies of weight fluctuation from 2-mm segment to 2-mm segments showed, however, that such assumptions are not entirely valid. This point will be examined later on.

Handling hair segments whose length can vary from 0.5 mm to 5 mm was in any case difficult with forceps, and could have been a major cause of contamination if the proper precautions were not taken. For these reasons, hair segments were picked up by a small capillary tube connected to an aspirator.. The diameter of the tube was small enough to prevent the entry of the sample. This device permitted the handling of very small hair samples (down to 0.5 mm).

2.4) Atomic Absorption Measurements

The natural copper and zinc contents of individual segments were measured by means of a model 305 Perkin-Elmer Atomic Absorption Spectrophotometer equipped with an HGA 70 carbon tube furnace. A three-cycle analytical procedure was used as recommended by the manufacturer: First, the drying step, which lasted 10 seconds, and in which the temperature was raised to 100 ^{o}C; then a 15-second ashing step, where the hair sample was charred at a temperature of 1100 ^{o}C; and the final step, where the temperature was raised to 2600 ^{o}C for 5 seconds, during which time the Cu and Zn contained in the sample were atomized. During the entire operation the carbon tube was flushed

with argon at a constant flow rate of 100 liters/minute to inhibit air-oxidation of the carbon at high temperature. This equipment permitted the analysis of solid hair samples without prior dissolution, which improved the detection limit for all the trace elements by perhaps a factor of 100, compared to the conventional flame method.

Another striking advantage of this technique is the simplicity and rapidity of each analysis, since after the hair sample had been cut, it just had to be transferred to the centre of the graphite furnace, which was achieved by means of a specially designed tantalum spoon. The very few steps during which each hair sample had to be manipulated may have been important in control of contamination, since further experiments, where each sample was dissolved in ultra-pure nitric acid, suffered some difficulty in producing consistent results. This point will be discussed later.

Despite the simplicity of each analysis, certain precautions still had to be taken in order to get consistent measurements. For example, the centering of the hair sample within the furnace was expected to be important, since the furnace temperature must vary from the center to the ends. The position of the sample after each insertion was therefore checked by visual inspection from the side orifice of the furnace, before and during the drying and ashing steps of each analysis. It was found that, especially during the ashing

step, the very light hair segments tended to be blown away from the center of the furnace by the stream of argon gas. This problem was alleviated by interrupting the argon gas flow during the two first stages of the analysis, and by using graphite tubes featuring a central region of increased diameter.

Calibration of the analysis was achieved by means of aliquots of standard solutions of cupric or zinc ions, prepared from a known weight of pure copper or zinc metal dissolved in nitric acid.

2.5) Experimental Tracer Technique

Results obtained on the natural content of copper and zinc in human hair (discussed later) suggested the possibility that these two elements (and perhaps others) could enter the hair entirely from external contamination, rather than being incorporated into the hair structure during growth.

Experiments were therefore conducted to measure the response of hairs to soaking in solutions of these two metals.

2.5.1) Inactive Tracer

Solutions of cupric ion were prepared from weighed quantities of pure copper metal dissolved in nitric acid. The solutions were

diluted to a concentration either of 0.1 or 1.0 mg/ml, and the pH was adjusted to 4.9 by addition of sodium hydroxide solution prepared from reagent grade NaOH pellets. This pH value has been shown (Bat. A66) to be close to that at which maximum copper absorption by human hair takes place. Human hairs were plucked and washed by the procedure outlined above. They were then soaked in the above cupric ion solutions for a specified length of time, and then washed again for five minutes in distilled, deionized water, five minutes in acetone, and five minutes in ether, in order to remove copper solution wetting the external hair surface. The hairs were next subjected to the cutting and Atomic Absorption Analysis procedure described earlier.

2.5.2) Radioactive Tracers

2.5.2.1) Solutions Containing One Tracer

Soaking hair samples in solutions containing radioactive ions is another way to measure the amount of the element absorbed from solution. The Cu radiotracer solutions were prepared from copper metal previously irradiated with thermal neutrons in the nuclear reactor of the University of Washington, Seattle, Washington, until a ^{64}Cu specific activity of 0.1 mci/mg was obtained. The irradiated copper metal was dissolved in nitric acid and the solution diluted with distilled, deionized water, to produce a copper concentration of 0.6 mg/ml. After adjustment of the pH to 4.9 as before, previously

washed hair samples were soaked in this solution for known periods of time.

After soaking, the hairs were again rinsed as described in the previous section (but for 1 minute per step, instead of 5 minutes in order to avoid an 'extraction' of the sorbed radioactive copper from the hair), cut into 2-mm segments by previously described techniques, and each individual segment was mounted on an aluminium planchette for radioactivity assay.

The total beta radioactivity was measured by means of a Si(Li) detector coupled to conventional electronic apparatus, the detector background being frequently monitored during the experiment. In order to convert the radioactivity measurements to mass of copper taken up per segment during the soaking operation, standard ^{64}Cu reference sources were periodically assayed during the experiment. Furthermore, this permitted the verification of the constancy of the detector's efficiency.

Similar experiments were carried out with solutions containing ^{65}Zn, ^{60}Co, and ^{76}As. The radioactive zinc (as zinc nitrate) was obtained from New England Nuclear Co., and had a reported specific activity of 3.68 mci/mg. The concentration and the pH were both adjusted to respective values of 0.1 mg/ml and 5.5. The radioactive cobalt (as cobalt chloride) was also obtained from New England Nuclear

Co., and had a reported specific activity of 0.1 mci/mg; the concentration and pH were adjusted to 0.29 mg/ml and 8.5 respectively. A precipitation of cobalt hydroxide was observed to be formed at this pH value. However, the absorption of cobalt from the residual solution onto the hair sample was found to be stronger than from solutions of a pH value of 3.5, where no precipitation was observed.

The radioactive arsenic was prepared from As_2O_3 irradiated at the University of Washington reactor, to a specific activity of 0.206 mci/mg. Shortly after the irradiation, the arsenic trioxide was dissolved in 15 ml. of 1 M NaOH, and the final concentration of arsenic was adjusted to 0.58 mg/ml and the pH to 5.5.

2.5.2.2) Solutions Containing Two Tracer Materials

The widely different quantities of various trace metals observed to be sorbed onto hairs suggested that studies of simultaneous absorption of two (or more) elements would be of interest. Such experiments would possibly reveal the existence of a competition process. Pairs of elements such as copper and mercury were first studied. Radioactive copper solution (as copper nitrate), which was supplied by New England Nuclear Corporation, with a reported specific activity of 0.25 mci/mg, was diluted to a concentration of 3.33 mg/ml. Six hundred µl of a radioactive mercury solution (as mercury nitrate, from New England Nuclear Corporation) with a specific activity of

5.13 mci/mg were added to the copper solution in order to obtain a concentration of mercury of 9.91 mg/ml. Finally the pH of the mixed solution was adjusted to a value of 6.5.

The experimental procedure was mostly the same as described above. The total amount of beta radioactivity (Cu and Hg) sorbed on each hair segment was separated into that due to 12.8h ^{64}Cu and that due to 46.6d ^{203}Hg by assay at different times, first immediately after each 2-mm sample was cut, and again 6 days later when the copper activity would have completely decayed and the measured activity was due to Hg alone. The Cu activity was calculated by subtraction.

2.5.2.3) Solutions Containing Five Radiotracers

Hair samples were soaked in a solution containing radioisotopes of Zn, Co, Hg, Ag and Sb for various periods of time. The concentrations and specific activities were as follows:

Table II

Concentration and specific activity of the isotopes used in the multi-tracer soaking experiment

Isotope	Element Concentration	Specific Activity
^{203}Hg	0.0925 mg/ml	5.13 mci/mg
^{110m}Ag	0.0612 mg/ml	4.93 mci/mg
^{124}Sb	0.366 mg/ml	0.989 mci/mg
^{65}Zn	0.180 mg/ml	4.49 mci/mg
^{60}Co	0.146 mg/ml	83.06 mci/mg

Each of these five radioisotopes were supplied by New England Nuclear Corporation as nitrates for mercury and silver, and as chlorides for antimony, zinc, and cobalt. Adjustment of the pH to the value of 4.5 was achieved by adding 1 M sodium hydroxide and 0.1 M nitric acid solutions. At this pH value, a slight precipitation of cobalt hydroxide was observed, but it has been shown that optimum absorption of these elements into hairs occurs at pH 4.5 and higher. In order to be sure that the measurements of absorption were valid, the precipitate was allowed to sediment so that the hair sample was only in contact with the clear supernatant solution.

The experimental procedure followed was then identical to what has been previously described, except for the activity measurements for which γ-spectroscopy techniques were employed. A Ge(Li) detector, with a 2.5 to 3.0 kev resolution for the 1332 kev ray from ^{60}Co, was employed. The detector was calibrated in energy by means of a standard source containing all five radioisotopes investigated. Conventional electronic apparatus was employed and the data were stored in the form of 800-channel spectra.

Spectra from hair samples and from a source of known intensity, which was prepared from known initial quantities of the five radionucleides, were taken consecutively, in order to simplify corrections for radioactive decay. Due to the low efficiency of the Ge-Li detector, and the small amounts of radioactivity absorbed, it

was necessary to cut the hair samples into pieces of 5-mm length and to measure the spectra for periods of time of 30 minutes.

The spectra were analyzed for peak intensities on an IBM 370 computer by means of the computer programme Sampo (Rou. 69). Finally, the values obtained were converted to mass of element absorbed by means of comparison with the results of computer analysis of spectra from the standard source.

2.6) Ingestion Experiment

Ingestion of copper (under medical control[1]) was undertaken by two subjects to determine whether any detectable quantity of dietary copper enters the hair structure via the root during hair growth, or whether such metals enter hair exclusively by being carried out from the human body by sweat and absorbed by the proximal part of the hair shaft (Bat. B66, Lim. 66). Quantities of 20 and 40 mg of copper (as copper sulfate) were weighed out, dissolved in distilled water, and ingested respectively by subjects F and E over a period of 24 hours as four equal 6-hourly doses. The concentrations of the solutions used for ingestion were 0.101 mg/ml for subject E and 0.05 mg/ml for subject F.

1. The experiment of copper ingestion by two volunteer subjects was first approved by the University Committee for Human Experimentation, and supervised by Dr. Lipinski. Medical exams were performed on both subjects by Dr. Lawton before and after the copper ingestion, as were analysis of blood copper levels.

Head hair samples were plucked individually from the two subjects involved in this experiment, and were subjected to the washing and Atomic Absorption Analysis previously described.

The times with respect to the ingestion at which the hairs were taken are given (together with the dates on which the subjects washed their hair) in Tables IX and X , since this last factor appeared to be highly relevant in connection with redistribution of the copper absorbed in hairs.

In order to find possible correlations between the ingestion of copper and the concentration of this element in the blood serum as a possible intermediary in transport to the hair, samples of blood were periodically taken from both subjects, before and after the copper ingestion at times given in tables IX and X respectively. Analysis for copper concentration in blood serum was done at Simon Fraser University by energy dispersive X-Ray Fluorescence Spectrometry, and by Atomic Absorption in an independent laboratory (B.C. Bio-medical Laboratories Limited). The X-ray measurements were done by Mr. I. G. Stump, under the supervision of Dr. J. D'Auria, to whom we are most grateful.

3) RESULTS

3.1) Experiments on Copper

3.1.1) Results for Indigenous Copper in Hair

Figure 2 shows the mass of copper found (and the corresponding calculated copper concentration) in 2- or 5-mm segments of individual human hairs, as a function of the distance of the segment from the hair root. Data in each part of the figure are for several hairs plucked from randomly located sites on the head of a single subject, and all verified as being in the anagen phase of the growth cycle at the moment of plucking. The seven subjects concerned in the several parts of this figure are described in Table III. It is seen in Figures 2a to 2g that hairs from a single individual exhibit generally similar copper patterns with the exception of subject H, whose hair has been submitted to a bleaching treatment. In the case of some subjects such as B, C, D, E, and F, the concentration patterns shown in Fig. 2 are typical of the much greater volume of data obtained. The remainder are not shown, to avoid crowding the figure.

In order to determine quantitatively the significance of the similarity between the copper concentration pattern of hairs taken from a single individual, coefficients of correlation[2] have been calculated for pairs of patterns among those measured for the seven subjects concerned in Fig. 2.

2. See Appendix A.

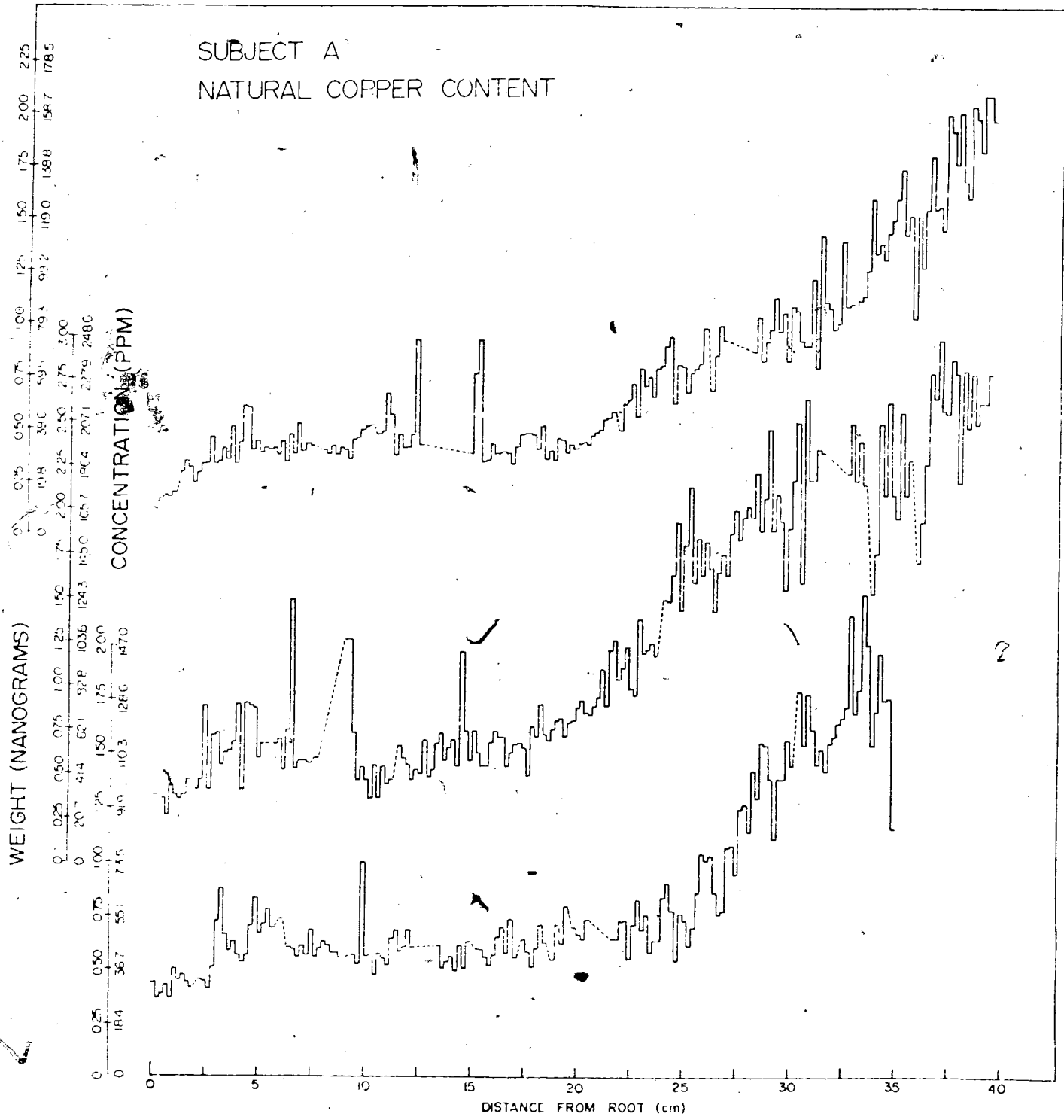

SUBJECT A
NATURAL COPPER CONTENT
WEIGHT (NANOGRAMS)
CONCENTRATION (PPM)
DISTANCE FROM ROOT (cm)
0
5
10
15
20
25
30
35
40

FIGURE 2a

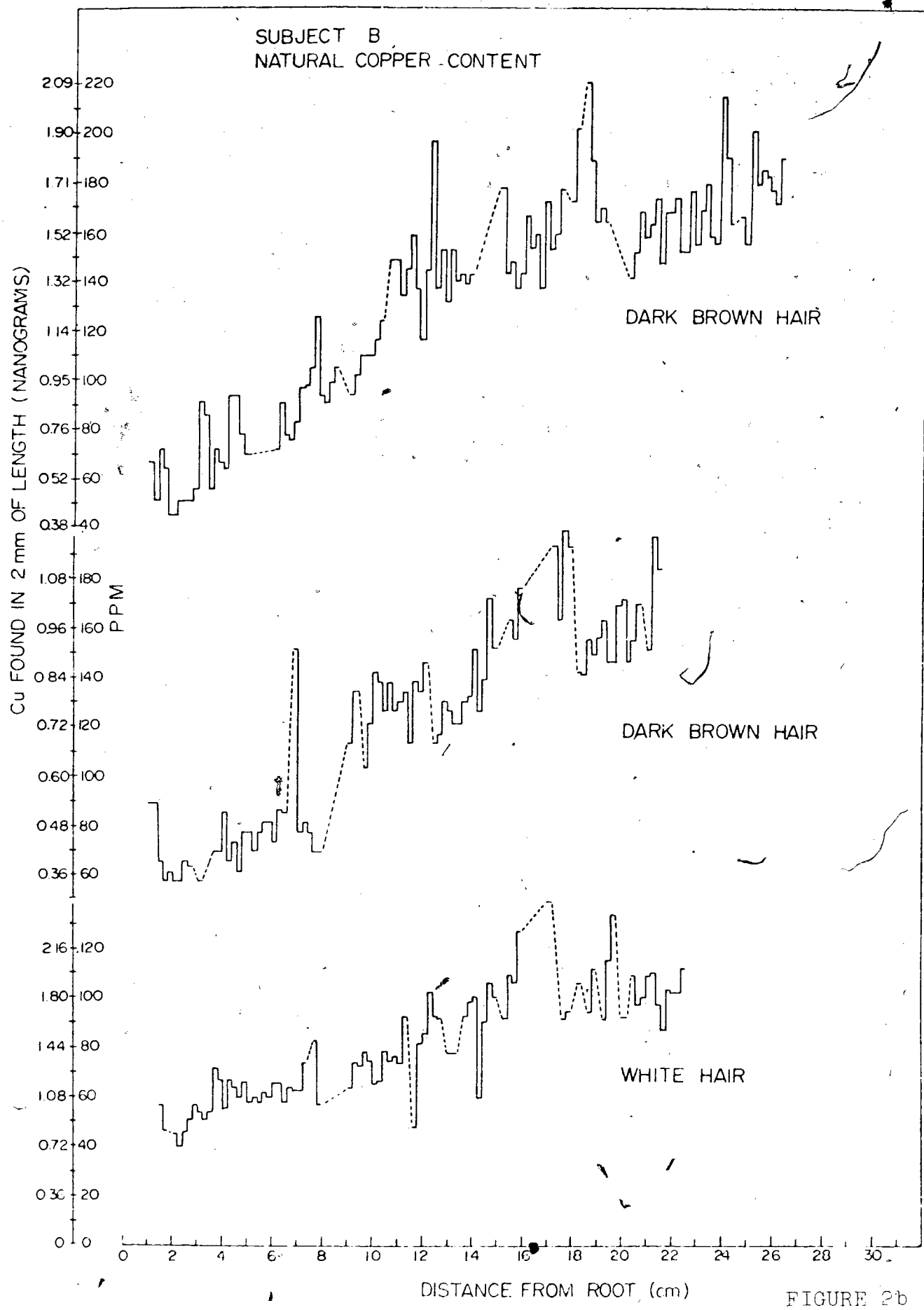

SUBJECT B
NATURAL COPPER CONTENT
DARK BROWN HAIR
DARK BROWN HAIR
WHITE HAIR
Cu FOUND IN 2 mm OF LENGTH (NANOGRAMS)
PPM
DISTANCE FROM ROOT (cm)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
0 20 40 60 80 100 120
0.36 0.48 0.60 0.72 0.84 0.96 1.08
60 80 100 120 140 160 180
0.38 0.52 0.76 0.95 1.14 1.32 1.52 1.71 1.90 2.09
40 60 80 100 120 140 160 180 200 220

FIGURE 2b

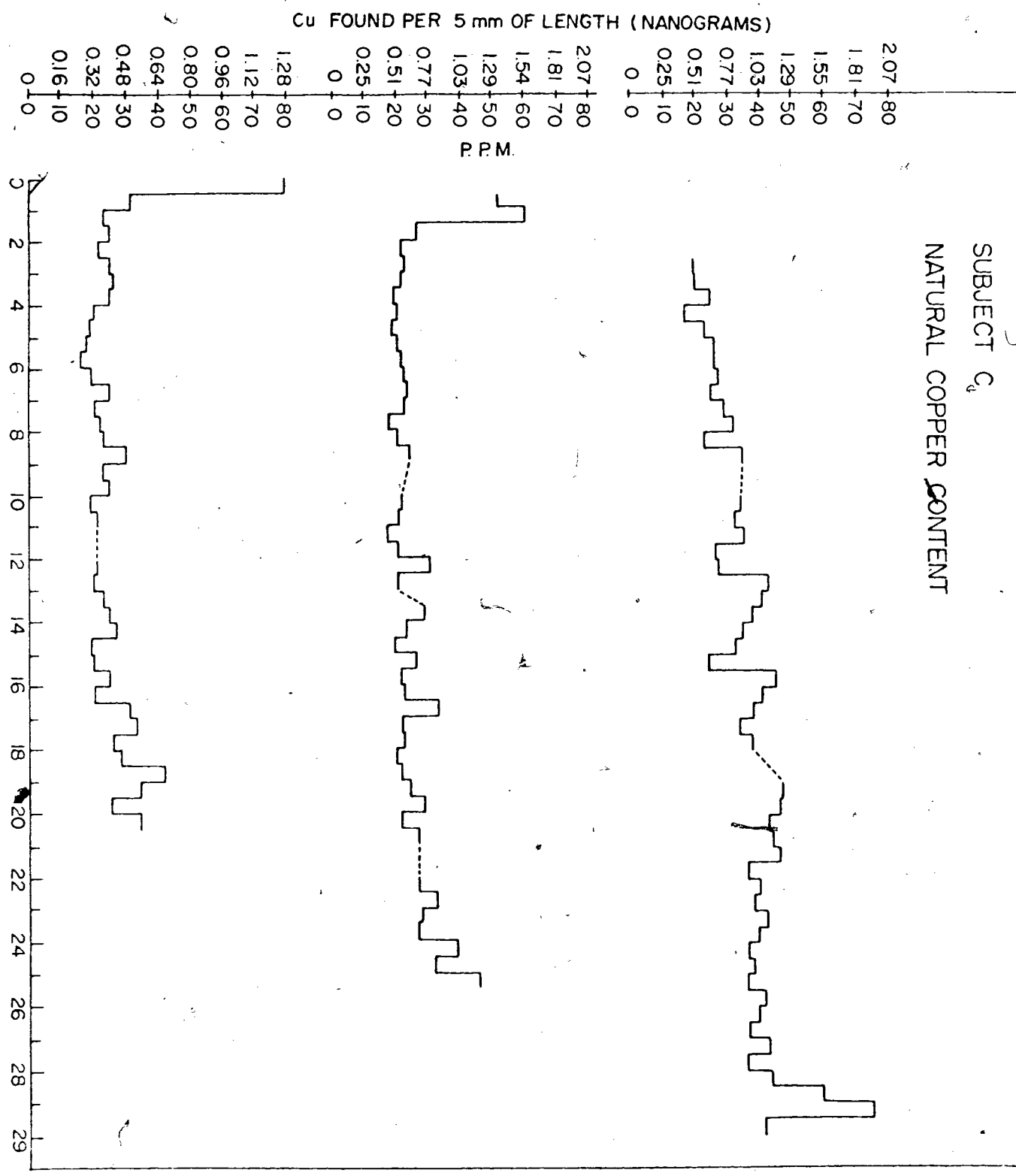

SUBJECT C4
NATURAL COPPER CONTENT
Cu FOUND PER 5 mm OF LENGTH (NANOGRAMS)
P.P.M.
0 0 0 0 0 0
0 10 0.25 10 0.16 10
0 20 0.51 20 0.32 20
0 30 0.77 30 0.48 30
0 40 1.03 40 0.64 40
0 50 1.29 50 0.80 50
0 60 1.55 60 0.96 60
0 70 1.81 70 1.12 70
0 80 2.07 80 1.28 80
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 29
DISTANCE FROM ROOT (cm)

FIGURE 2c

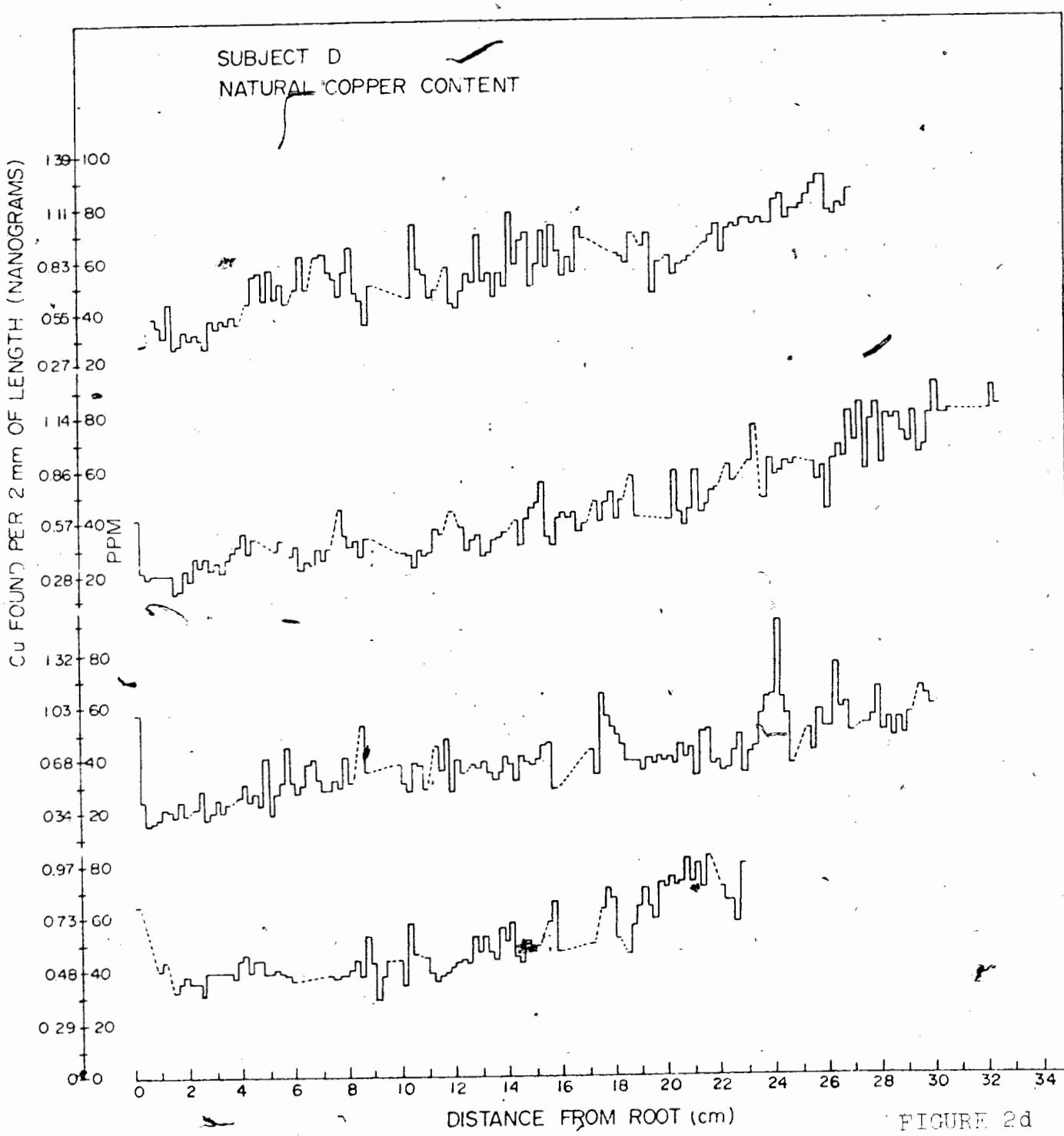

SUBJECT D
NATURAL COPPER CONTENT
Cu FOUND PER 2 mm OF LENGTH (NANOGRAMS)
PPM
1.39 100
1.11 80
0.83 60
0.55 40
0.27 20
1.14 80
0.86 60
0.57 40
0.28 20
1.32 80
1.03 60
0.68 40
0.34 20
0.97 80
0.73 60
0.48 40
0.29 20
0 0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
DISTANCE FROM ROOT (cm)

FIGURE 2d

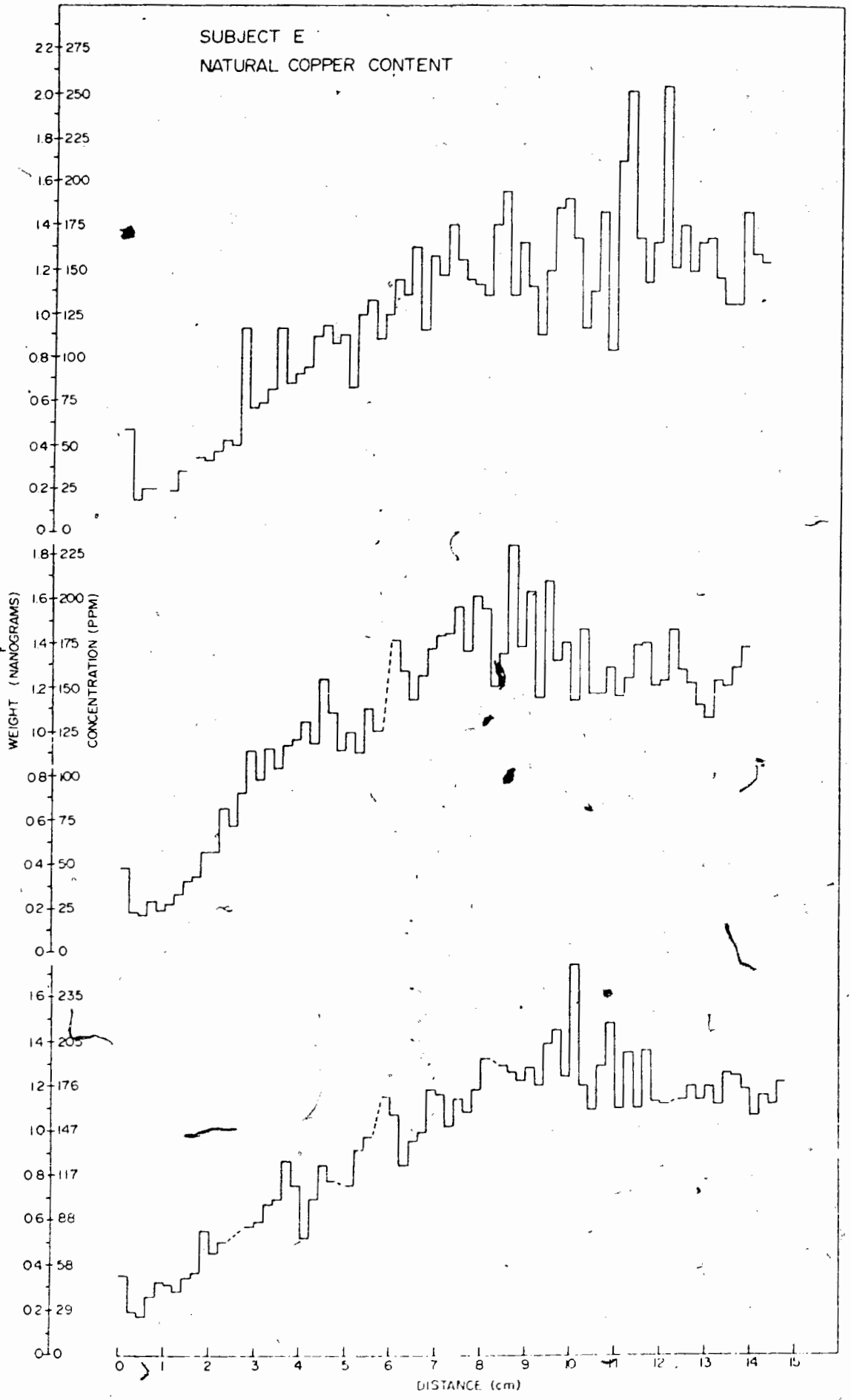

SUBJECT E
NATURAL COPPER CONTENT
WEIGHT (NANOGRAMS)
CONCENTRATION (PPM)
DISTANCE (cm)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
2.2 275
2.0 250
1.8 225
1.6 200
1.4 175
1.2 150
1.0 125
0.8 100
0.6 75
0.4 50
0.2 25
0 0
1.8 225
1.6 200
1.4 175
1.2 150
1.0 125
0.8 100
0.6 75
0.4 50
0.2 25
0 0
1.6 235
1.4 205
1.2 176
1.0 147
0.8 117
0.6 88
0.4 58
0.2 29
0 0

FIGURE 2e

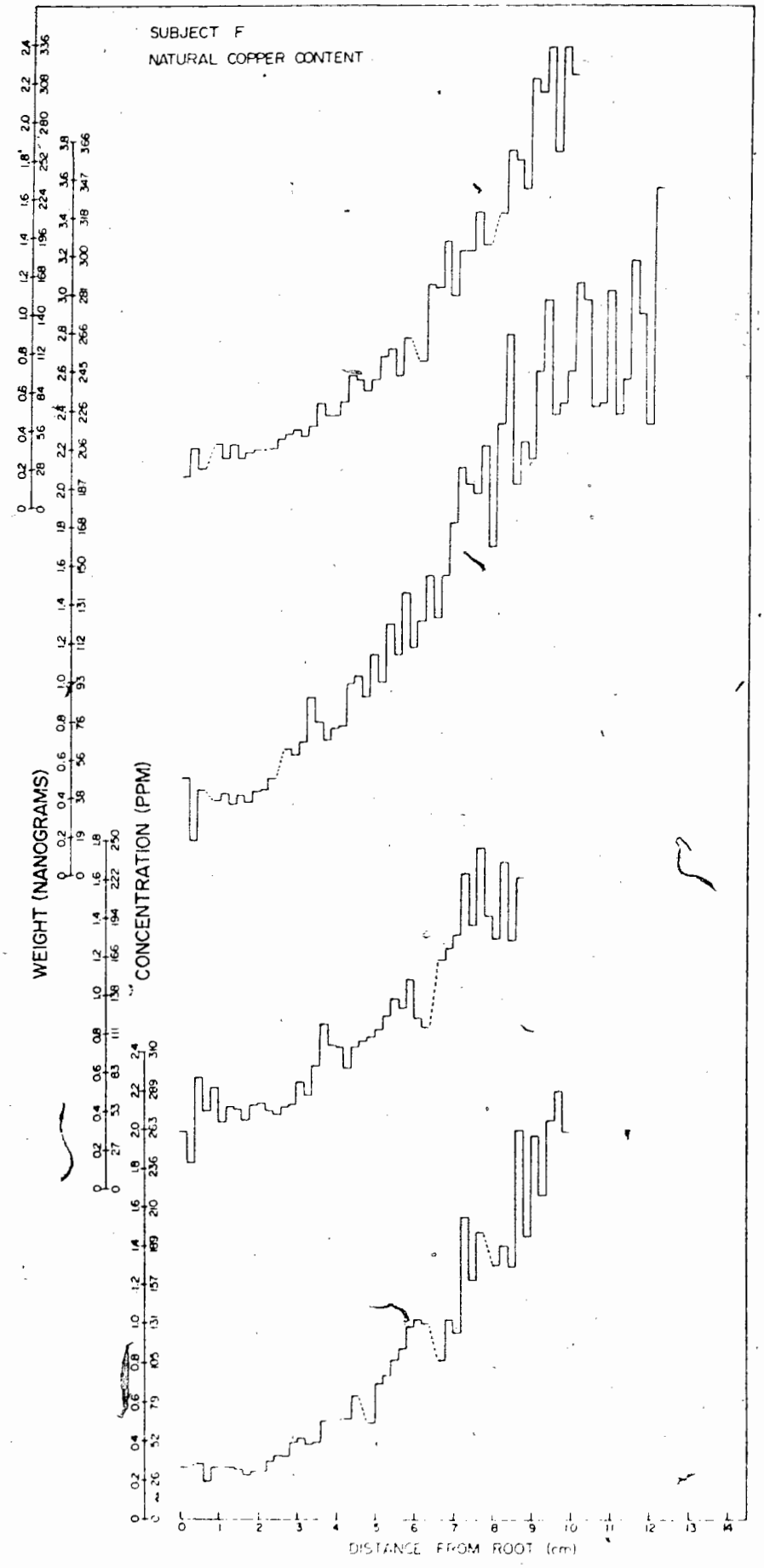

SUBJECT F
NATURAL COPPER CONTENT
WEIGHT (NANOGRAMS)
CONCENTRATION (PPM)
DISTANCE FROM ROOT (cm)

FIGURE 2f

NATURAL COPPER CONTENT SUBJECT:H

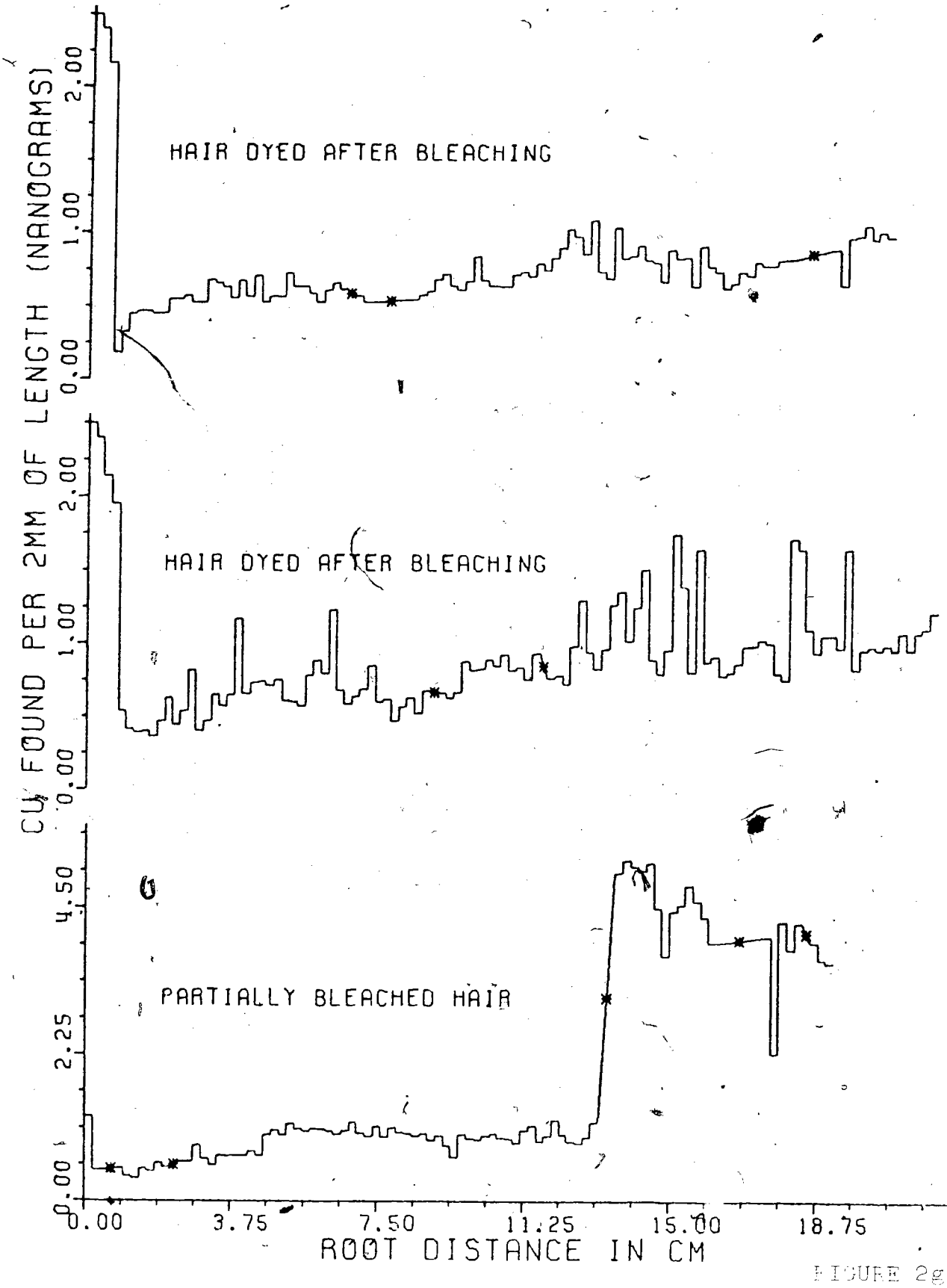


FIGURE 2g

TABLE III

Subjects from whom hair samples were analysed

Subject	Age	Sex	Domicile	Hair Colour
A	21	female	Vancouver, B. C.	Light brown
B	43	female	Vancouver, B. C.	Dark brown or black changing to white or grey
C	18	female	Paris, France	Blond
D	25	female	Vancouver, B. C.	Medium brown
E	31	male	Vancouver, B. C.	Light brown
F	30	male	Vancouver, B. C.	Dark blond
G	22	female	Vancouver, B. C.	Brown, partially bleached
H	24	female	Vancouver, B. C.	Brown, partially bleached
I	25	female	Vancouver, B. C.	Dark brown
J	29	female	Vancouver, B. C.	Light brown, partially bleached

The means of the distribution of the correlation coefficient values obtained for each subject are given in Table IV, together with the standard deviation of the distribution, and also the number of samples of which the concentration patterns have been used in the calculation.

The consistently high values obtained for those coefficients of correlation reinforce the impression (obtained by visual inspection) of similarity which is conveyed by the copper concentration patterns for hairs taken from a single individual (except for subject H, from whom the lower correlation coefficient reflects the dissimilarity between H_1 and H_2, H_3). In contrast with this resemblance it can be seen that hairs taken from different individuals differ in average Cu content (as expected from previous data on the distribution of average Cu contents of hairs from a general population (Per. 65)) and also in the magnitude of features such as slopes and local concentration fluctuations. A comparison of the copper patterns found in hairs taken from three different subjects is to be found in Fig. 3.

This difference between the characteristic patterns of the several subjects involved in this experiment can be again measured quantitatively by the coefficient of correlation, whose values are described in Table V. These were calculated from concentration patterns for pairs of hairs taken from two different subjects. However, because hairs of subject C were analyzed via segments 5 mm

TABLE IV

Correlation coefficient between the natural copper concentration patterns of each subject

Subject	Number of Samples	Mean of Correlation Coefficient	Standard Deviation
A	3	0.931	0.0064
B	3	0.935	0.023
C	3	0.882	0.001
D	3	0.879	0.007
E	5	0.914	0.002
F	3	0.949	0.030
H	3	0.412	0.502

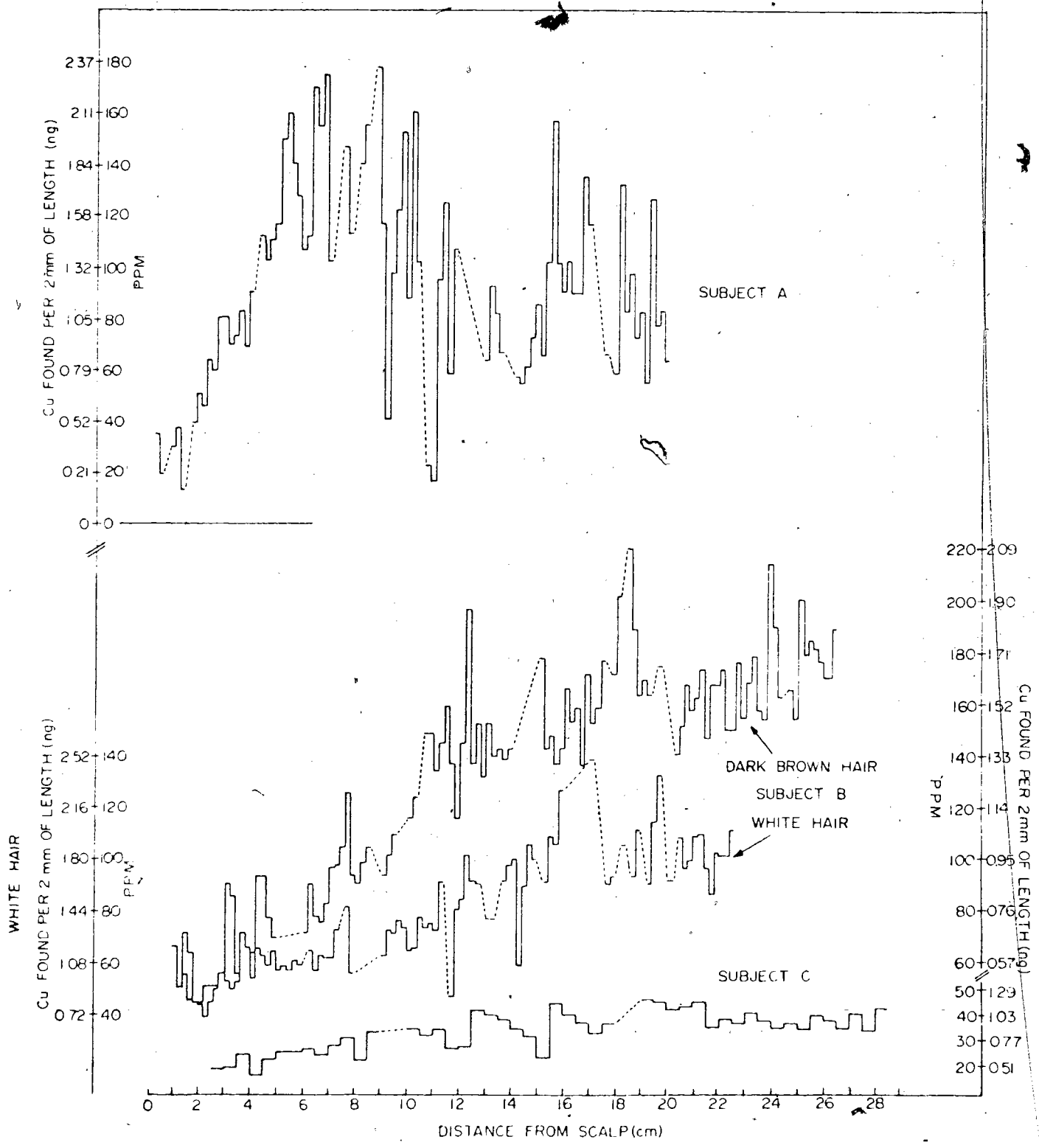

237 180
211 160
184 140
158 120
132 100
105 80
079 60
052 40
021 20
0 0
Cu FOUND PER 2mm OF LENGTH (ng)
PPM
SUBJECT A
220 209
200 190
180 171
160 152
140 133
120 114
100 095
80 076
60 057
50 129
40 103
30 077
20 051
Cu FOUND PER 2mm OF LENGTH (ng)
PPM
DARK BROWN HAIR
SUBJECT B
WHITE HAIR
WHITE HAIR
252 140
216 120
180 100
144 80
108 60
072 40
Cu FOUND PER 2 mm OF LENGTH (ng)
PPM
SUBJECT C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DISTANCE FROM SCALP (cm)

TABLE V

Correlation coefficients between the natural copper concentration patterns of different subjects

Subjects Whose Patterns Are Compared	Number of Samples Compared	Mean of Correlation Coefficient	Standard Deviation
A and B	9	0.856	0.058
A and D	9	0.797	0.074
A and E	15	0.805	0.067
A and F	9	0.670	0.095
B and D	9	0.917	0.026
B and E	15	0.829	0.027
B and F	9	0.832	0.041
D and E	15	0.782	0.12
D and F	9	0.643	0.124
E and F	15	0.867	0.045

long (instead of the usual 2 mm) it was not possible via the formalism developed in this work to compare the concentration patterns obtained for this subject with any other patterns shown so far. The results obtained are generally lower than the means of the correlation coefficients for hairs from a single individual.

The significance of the difference between the two sets of data can be assessed by the student's t-test. Table VI shows the t-values obtained for all possible combinations of correlation coefficients taken from Tables IV and V, and indicates that the probability of recognising, for example, two hairs as coming from two different subjects which in fact come from the same individual, is less than 3%.

In some cases, this probability drops down to less than 10^{-5} (as for the comparison between the correlation coefficient of subject E patterns with the correlation coefficients calculated between the patterns of subjects E and B), which makes an identification by this method relatively less ambiguous.

In one case, however, the patterns in hairs from subjects B and D are more correlated than the patterns in hairs from subject D alone. This circumstance (which occurs in only one case in twenty) is due to the low correlation between the patterns of subject D combined with a general similarity between the patterns of subjects B and D.

TABLE VI

Student's t-test on the correlation coefficients

Set of Correlation Coefficients Being Compared	Degree of Freedom (n_1+n_2-2)	t	α
A+B and A	10	2.16	0.03
A+B and B	10	2.28	0.025
A+D and A	10	2.99	0.006
A+D and D	10	1.83	0.05
A+E and A	16	3.15	0.003
A+E and E	18	3.59	0.001
A+F and A	10	4.61	0.0005
A+F and F	10	4.87	0.0003
B+D and B	10	1.06	0.16
B+D and D	10	r(B+D) > r(D)	
B+E and B	16	6.32	1×10^{-5}
B+E and E	18	6.91	$< 1\times10^{-5}$
B+F and B	10	4.06	0.0011
B+F and F	10	4.56	0.0005
D+E and D	16	1.36	0.090
D+E and E	18	2.42	0.013
D+F and D	10	3.19	0.004
D+F and F	10	4.11	0.001
E+F and E	18	2.33	0.016
E+F and F	16	3.00	0.004

However, the availability (say via multi-element AA) of pattern data for several elements in the same hair would be expected to allow compounding of probabilities such as the above, to permit a more certain matching of hairs from the same source.

3.1.2) Origin of Pattern Features

All the copper patterns measured, for these subjects and others, show three general characteristics:

- ere is a fluctuation of the copper content between adjacent segments by as much as a factor of 2.

- there is a minimum copper concentration at about 1-2 cm from the root, and there is a generally increasing concentration of copper with increasing distance from the root.

- ere are, superimposed on this general trend, regions of the hair extending for several centimeters where the copper concentration is much higher (or lower) than in adjacent regions, and appear to be characteristic of a given individual.

These features could perhaps be rationalized in the following terms:

1) The observed segment-to-segment fluctuations might be due to faulty analytical technique or to varying external contamination which was not completely removed by the washing procedure employed.

2) The increasing concentration from the root to the distal end might be due to absorption of copper from the environment, which had proceeded to a greater extent with the longer total exposure time to which the distal end had been subjected. It can be seen, however, that this feature is not common to every sample for which the patterns are shown in Fig. 2. In particular, the hairs of subject C show no evidence of such an increase of copper concentration, which already raises some question regarding the above interpretation.

3) The region of locally increased or decreased copper concentrations might reflect changes in the dietary copper content, which might in turn evidence corresponding changes in the blood copper level, and hence the extent of incorporation of copper into the hair growing from the subject's follicles.

1.3) Validity of the Analysis

Question 1 above, on the validity of the present analytical technique, is clearly of utmost importance to the significance of the

present research work as a whole. It seemed critical, therefore, to scrutinise the techniques employed.

The question first arises as to whether the values obtained by Atomic Absorption Analysis are accurate, since duplicate analyses of the same are impossible because of the destructive character of the method, and because we have seen previously that this method is not free from interferences. In particular, the calibration of the absorption signal measured during the atomization of a solid hair sample by comparison with the signal obtained from an aliquot of a standard solution could be a source of error.

First therefore, a comparison was attempted between hair segments analysed in the solid phase as described above, and larger segments of another hair from the same subject which were subjected to a prior dissolution in concentrated nitric acid, aliquots of the resulting solution being pipetted into the furnace.

Several problems affect such a comparison. It would have been preferable to compare hair segments of identical composition. Since these evidently do not exist, the measured average copper values and the patterns from two hairs from the same subject were compared.

Figure 4 shows the results that were obtained with hairs from subject D. The close similarities of the general patterns are to be

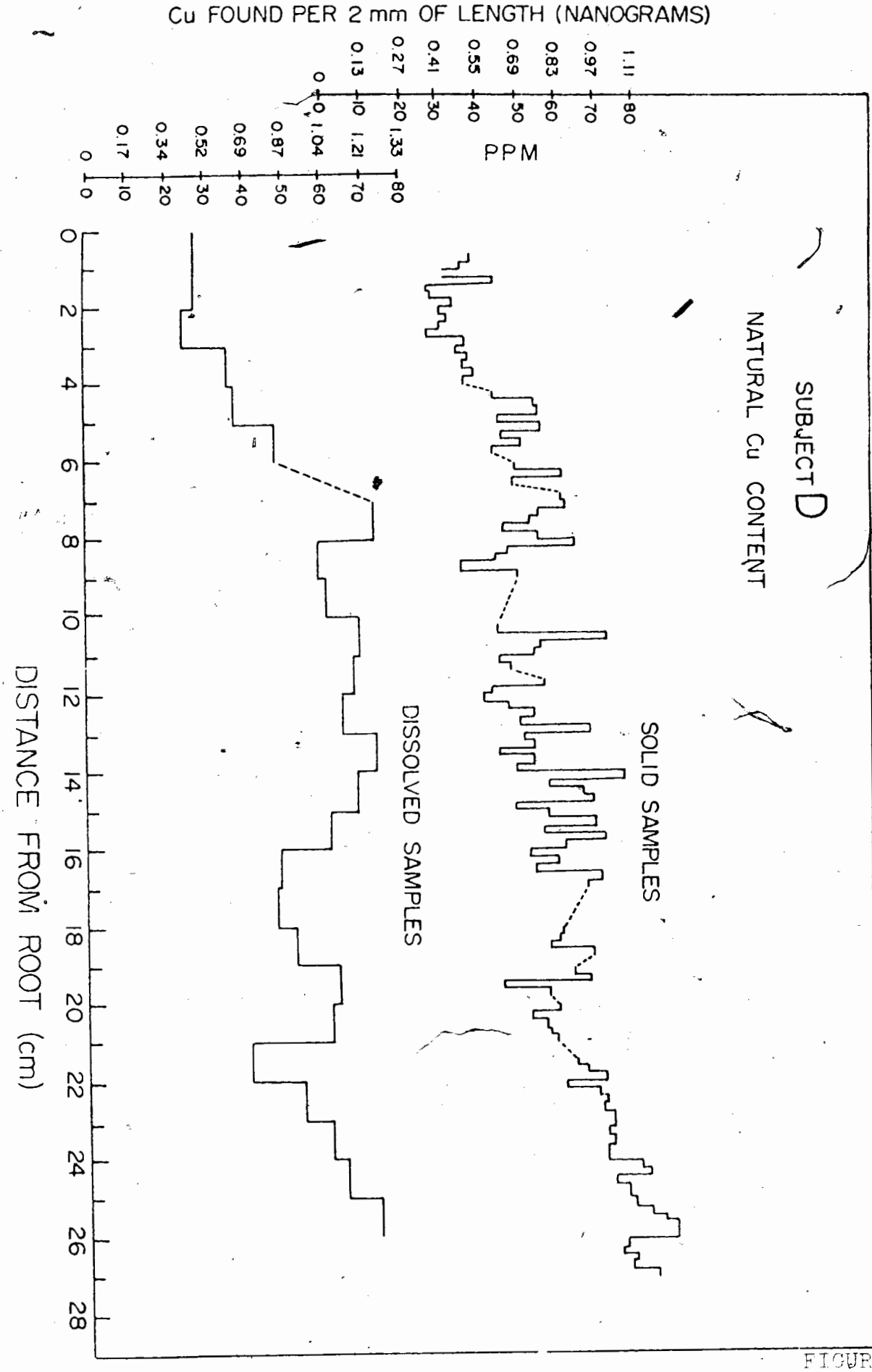

Cu FOUND PER 2 mm OF LENGTH (NANOGRAMS)
0 0.13 0.27 0.41 0.55 0.69 0.83 0.97 1.11
0 10 20 30 40 50 60 70 80
PPM
0 1.04 1.21 1.33
0 0.17 0.34 0.52 0.69 0.87
0 10 20 30 40 50 60 70 80
SUBJECT D
NATURAL Cu CONTENT
SOLID SAMPLES
DISSOLVED SAMPLES
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DISTANCE FROM ROOT (cm)

FIGURE 4

noted, and the data on the average concentration values measured along these two hairs indicate that analysis of samples in the solid phase and after dissolution give the same copper concentration value to within 10%. The tendency for the dissolved samples to have a slightly higher concentration may reflect contamination with copper from the very pure nitric acid employed. In the dissolved samples, the copper is expected to have generally the same chemical form and environment as in the standard solution, which is made up from copper metal dissolved in nitric acid. Thus, the similarities of values found between solid and dissolved samples is a good indication that nearly the same proportion of copper atoms (presumably close to 100%) are liberated from the solid samples and from the standard solution.

Further verification of the accuracy of the results was attempted by checking the influence on the analytical results of proteinaceous material present in the furnace during analysis. This was achieved by analysing a sample of tyrosinase (Sigma Laboratories), which had a reported copper concentration of about 300 ppm, sucessively by Atomic Absorption ~~both on a~~ solid and a dissolved sample, and secondly by X-Ray Fluorescence Analysis on the evaporated residue of the solution and on a solid sample. The latter method of analysis is presumably free of any interferences due to protein material to which Atomic Absorption may be subject. Calibration in both cases was achieved by comparison with aliquots of a standard copper solution or with a standard N.B.S. orchard leaves material of known Cu content (Sta. 72)

where the undissolved tyrosinase was analysed by X-Ray Fluorescence. The results are shown in Table VII and indicate an agreement consistent with the estimated 10% error of the Atomic Absorption Technique, given possible inhomogeneity of the protein material, when analysed undissolved by X-Ray Fluorescence.

It seems, in view of the consistency of the results obtained so far, that the measured values and the variations of copper concentrations along the hair samples analysed are reasonably accurate.

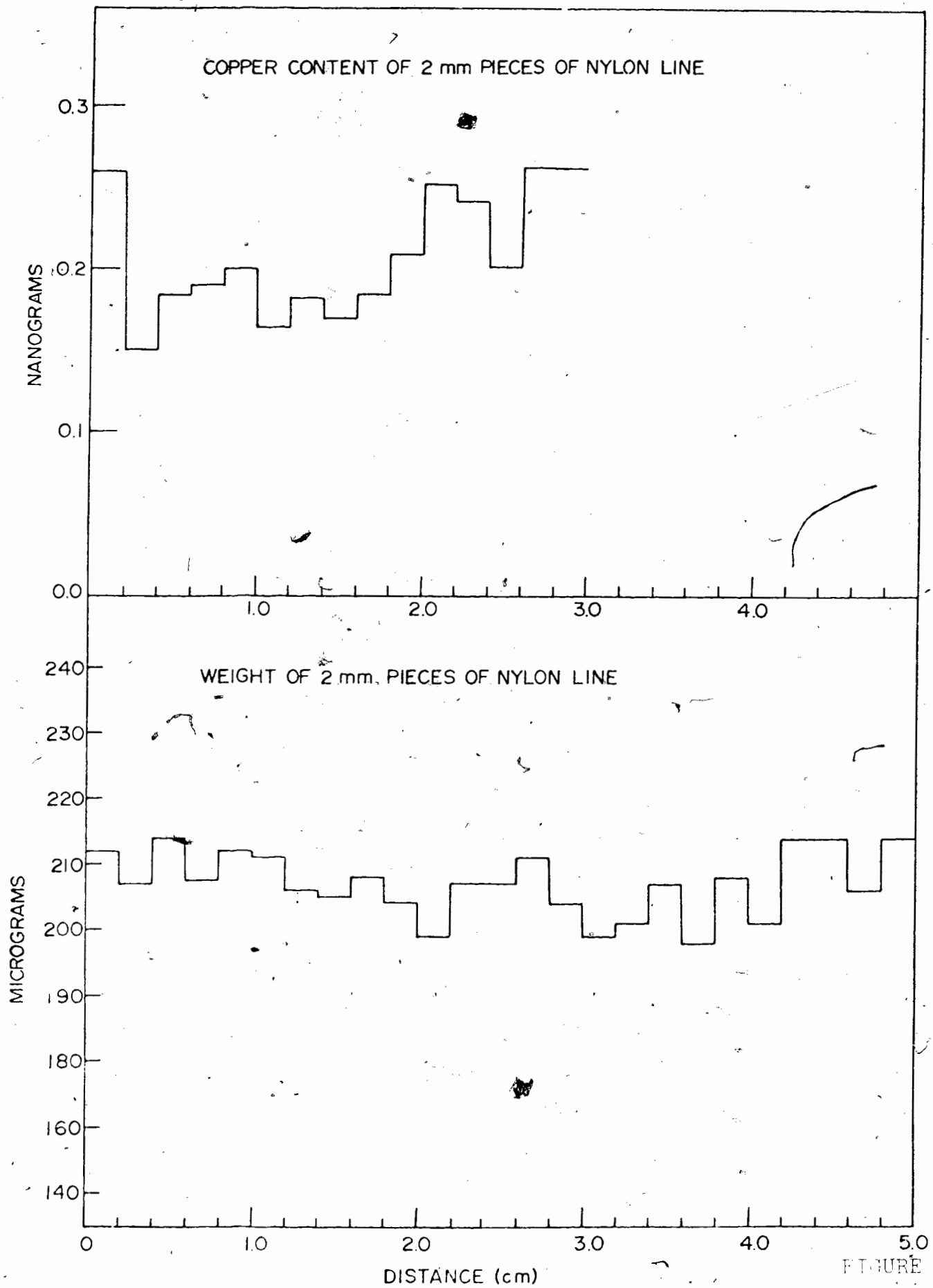
Next, to check whether the segment-to-segment variations, which seem to be a common characteristic of every hair sample analysed by this method, were due to variable contamination of each segment during the analysis due to poor handling technique, it was decided to analyse, by the same procedure, 2-mm segments of a nylon monofilament fishing line, which is known to have a fairly homogeneous and amorphous structure. This feature might be expected to lead to the copper content of each segment analysed being fairly constant, unless the method of handling and analysis adopted so far is the real cause for a segment-to-segment variation.

The results shown in Fig. 5 clearly show a very much reduced Cu concentration from that observed in hair, and demonstrate that the segment-to-segment copper content variations are smaller (± 18%) than

TABLE VII

Analysis of Cu-content of Tyrosinase via atomic absorption spectrometry and X-ray fluorescence spectrometry (precision ± 10%)

	XRF	AA
dissolved samples	302 ppm	273 ppm
solid samples	382 ppm	288, 338, 285 ppm


COPPER CONTENT OF 2 mm PIECES OF NYLON LINE
NANOGRAMS
0.3
0.2
0.1
0.0
1.0
2.0
3.0
4.0
WEIGHT OF 2 mm PIECES OF NYLON LINE
MICROGRAMS
240
230
220
210
200
190
180
160
140
0
1.0
2.0
3.0
4.0
5.0
DISTANCE (cm)

FIGURE

those generally observed with hair. Thus, if the analysis of a filament, whose homogeneous structure is not expected to lead to a variation of copper concentration from segment-to-segment, has produced more constant results than has been obtained with hair fibers with known structural inhomogeneity, it is possible to conclude that the piece-to-piece variation observed with hairs was not due to contamination during the analysis to a significant extent. Thus it was presumably due either to the natural inhomogeneity of the copper content of the hair shaft, or perhaps to superficial contamination present before the analysis began.

3.1.4) Effects of Hair Washing

The importance of hair washing as a means of removing such contamination has been emphasized earlier. It was evidently necessary to select a washing technique, and to determine an optimum washing time sufficient to remove the superficial contamination of hair, but not the trace elements bound to the hair structure proper. It was decided to employ one of the previously described washing procedures (Obr. 72), rather than to introduce another.

Three hairs drawn from the head of a single subject (subject A)[3] were separately subjected to the washing sequence described earlier

3. The three hairs used in this experiment were taken 9 months before the three hairs whose patterns are represented in Fig. 2_A, which explains the dissimilarity between the two series of patterns.

(see section 2.2), but with times of 5, 10, and 15 minutes per step of the procedure. The patterns measured after such washing process are shown in Fig. 6.

It is seen that the length of the washing time influences the magnitude of the features of the observed copper concentration pattern, but that even after 15 minutes of washing per step, such features are still visible. The highly erratic concentration variations obtained with hairs which were not subjected to any washing, had, however, dissappeared after a washing time of five minutes per step. Thus, this duration appeared to be a reasonable compromise between removing contamination on the one hand, and avoiding the washing out of a potentially significant concentration pattern on the other.

The persistence of pattern features even after the 15 minutes per step washing, plus the apparent validity of the analytical method, suggest that the concentration fluctuations are real (and not an analytical artifact) and may be associated with variations in the hair structure (rather than with surface contamination).

Another such quantity may be the density of the hair material, since the segment-to-segment fluctuation of copper content observed may have resulted from variation of segment weight. Figure 7 shows the results of weighing sucessive 2- and 5-mm segments of a hair from

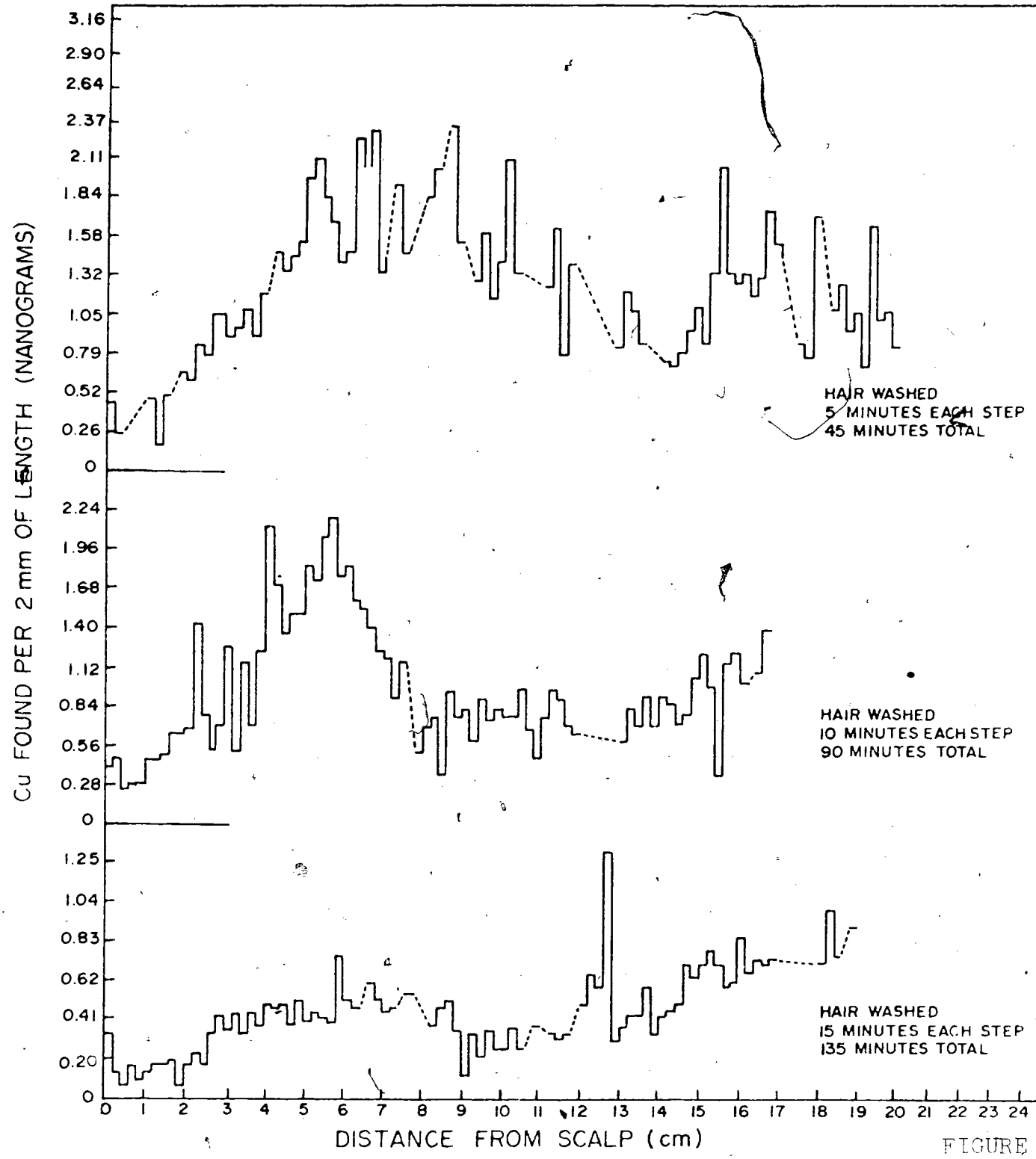
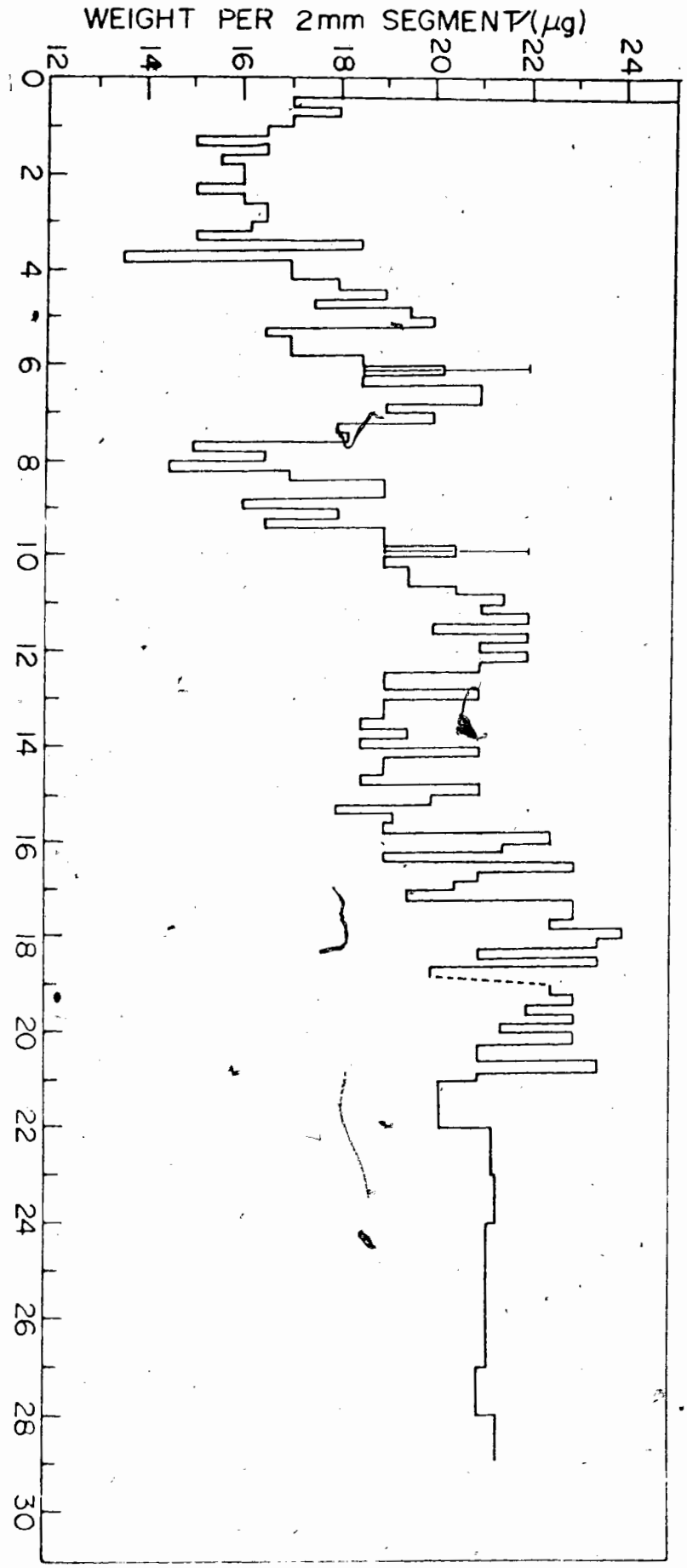

Cu FOUND PER 2 mm OF LENGTH (NANOGRAMS)
3.16
2.90
2.64
2.37
2.11
1.84
1.58
1.32
1.05
0.79
0.52
0.26
0
HAIR WASHED
5 MINUTES EACH STEP
45 MINUTES TOTAL
2.24
1.96
1.68
1.40
1.12
0.84
0.56
0.28
0
HAIR WASHED
10 MINUTES EACH STEP
90 MINUTES TOTAL
1.25
1.04
0.83
0.62
0.41
0.20
0
HAIR WASHED
15 MINUTES EACH STEP
135 MINUTES TOTAL
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
DISTANCE FROM SCALP (cm)

FIGURE 6


WEIGHT PER 2mm SEGMENT (μg)
12
14
16
18
20
22
24
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DISTANCE FROM ROOT (cm)

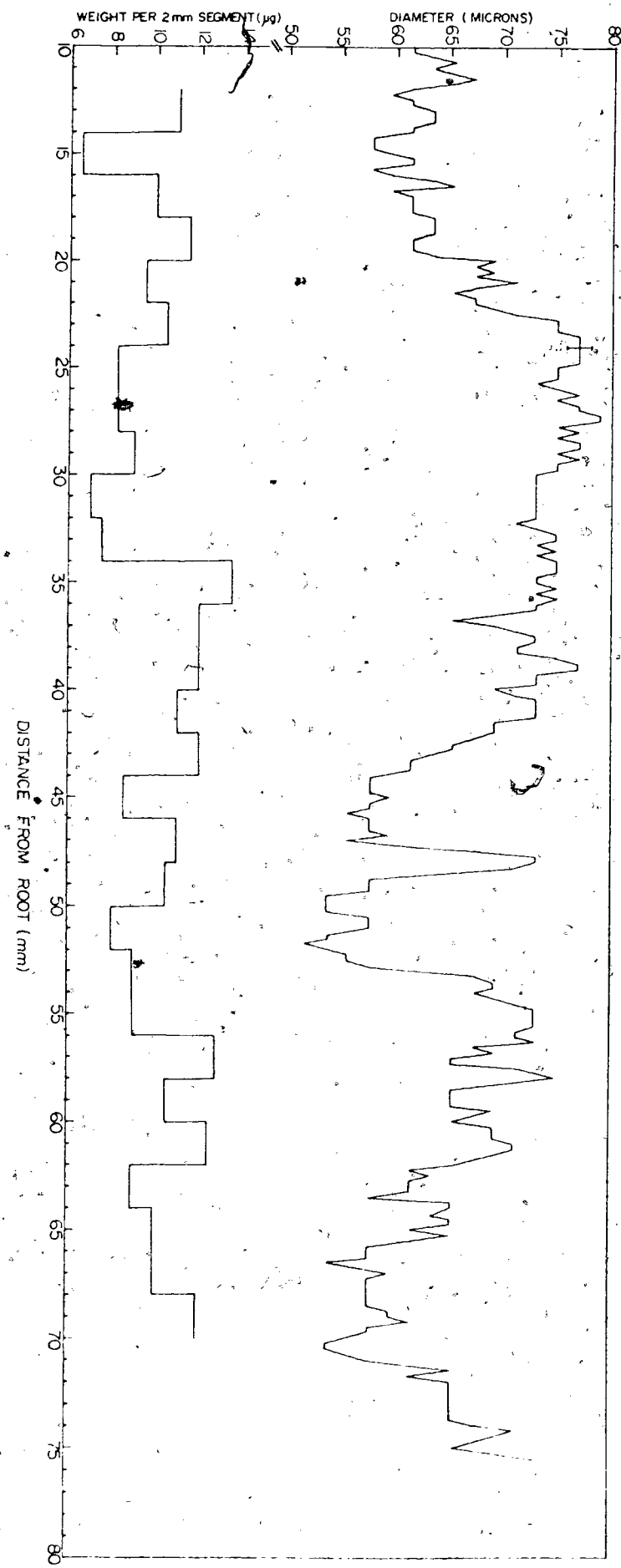
FIGURE

subject A, together with the estimated precision of such measurements (7%).

It can be seen that segment-to-segment weight fluctuations are far smaller in magnitude than the observed variation of copper content, since the maximum fluctuation in weight measured between two adjacent 2-mm segments was only 17% of the average mass (at a distance of 4 cm from the root).

Another feature which might be linked to the observed variation of copper content is the fluctuation of hair cross-section, and hence internal surface area available for ion absorption.

The diameter of a hair from subject A was measured microscopically at intervals along the hair much shorter (0.25 mm) than the 2 mm imposed otherwise by the necessity of analysing the copper content. The results are shown in Fig. 8 (with the estimated precision being 1.5%), together with the weight of the corresponding 2-mm segments. The cyclic diameter variations observed are presumably a reflection of the helical form of the hair and an eliptical cross-section (Pri. 74), and are not reflected in a corresponding cyclic variation of hair weight. Beyond such variations, however, there are local points of significantly reduced and increased diameter, but which are too small in magnitude to be the cause of the Cu content variation.


WEIGHT PER 2mm SEGMENT (µg)
DIAMETER (MICRONS)
DISTANCE FROM ROOT (mm)

FIGURE

It seems, then, that none of the possible causes of Cu content fluctuation investigated so far are in fact responsible for it alone, although they may be partial contributors.

3.1.5) Tracer Experiments

Certain features of the copper patterns observed in hairs, such as the increase of concentration from the root to the distal end, may perhaps, as suggested earlier, be rationalized in terms of longer exposure times to contamination. This was directly verified by soaking a hair in a copper solution under conditions such that the added metal was very much greater in quantity than the indigenous copper. This should result in a fairly constant copper concentration from one end of the hair to the other, following from the exposure time now being the same for all locations on the hair.

Likewise, if the regions of locally increased and decreased copper concentration are indeed, as suggested, due to dietary influences, then following such an intensive soaking operation such features should be submerged. Such expectations were subjected to direct test.

3.1.5.1) Inactive Tracer

Two sets of hairs plucked from the head of subject A were

subjected to a soaking in a 0.1mg/ml copper solution, adjusted to pH 4.5, for 1 and 26 hours respectively. The results are shown in Fig. 9, the concentration and time of soaking being indicated for each hair. Again, the patterns shown in this figure have been selected from a much larger collection of data, all displaying strictly similar features. Thus the following discussion is based on much more data than just that shown in the figure.

There is strong evidence that the copper taken up by the hair during the soaking is not absorbed uniformly along the length of the hair, whether the amount added is 10 or 100 times the content prior to soaking. Furthermore, the fact that after 26 hours of soaking the hair sample absorbed almost 5 times more copper than during 1 hour of contact with the same solution, could indicate that the absorption process could be partially governed by a phenomenon of diffusion whose slow rate may limit the amount of metal absorbed into the keratin.

The increase with distance in the natural copper content observed in Fig. 2 is again noticed for hair samples of subject A which have been soaked 1 hour as well as 26 hours in the copper solution. The extent of this variation can be compared with the increase of natural copper concentration in Fig. 10, where two hairs from subject B, which have been soaked in 0.1 mg/ml Cu^{++} solution for 3 hours, have their patterns reproduced, along with an untreated hair.

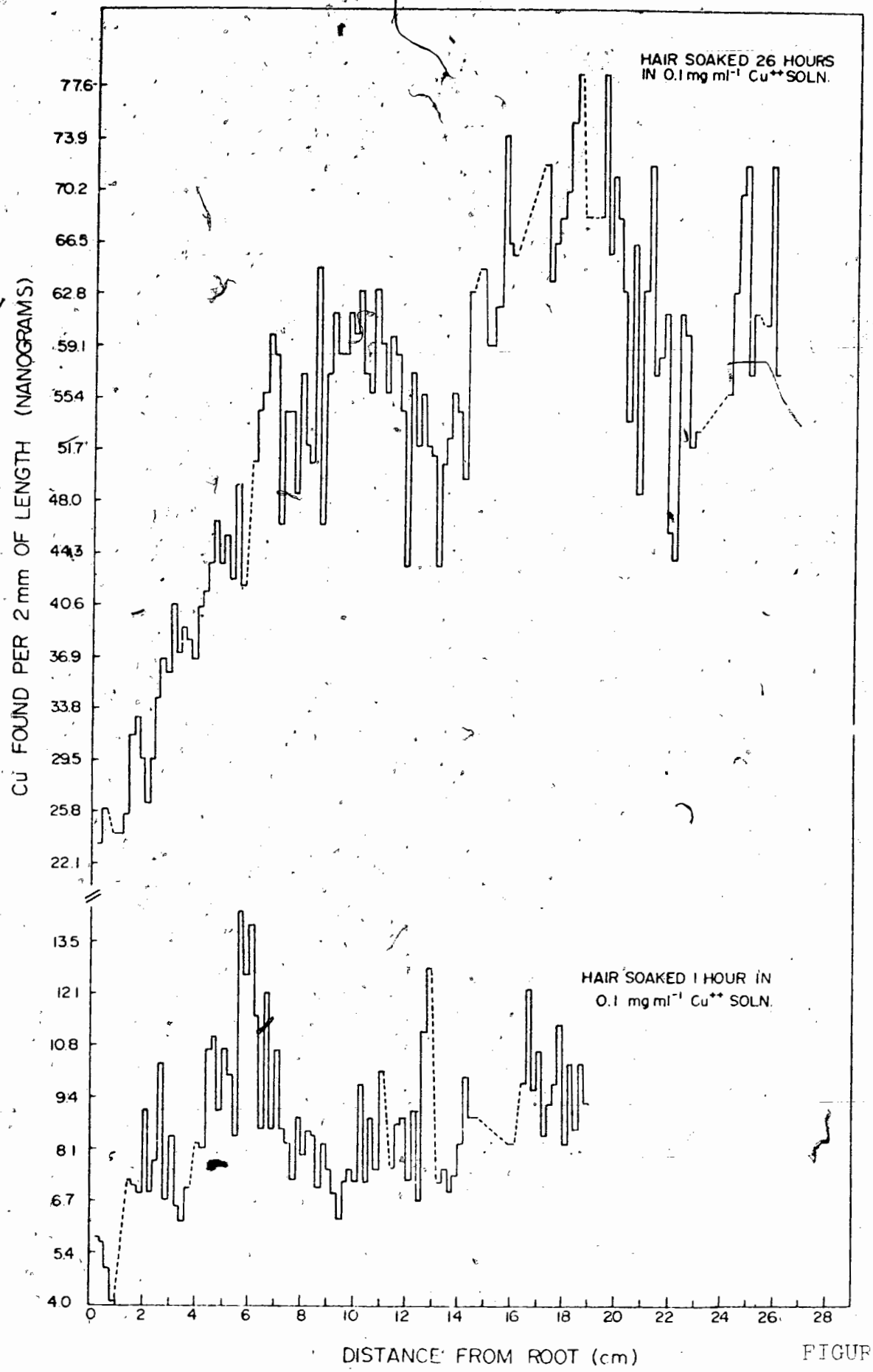

HAIR SOAKED 26 HOURS IN 0.1 mg ml⁻¹ Cu⁺⁺ SOLN.
HAIR SOAKED 1 HOUR IN 0.1 mg ml⁻¹ Cu⁺⁺ SOLN.
Cu FOUND PER 2 mm OF LENGTH (NANOGRAMS)
77.6
73.9
70.2
66.5
62.8
59.1
55.4
51.7
48.0
44.3
40.6
36.9
33.8
29.5
25.8
22.1
13.5
12.1
10.8
9.4
8.1
6.7
5.4
4.0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
DISTANCE FROM ROOT (cm)

FIGURE 9

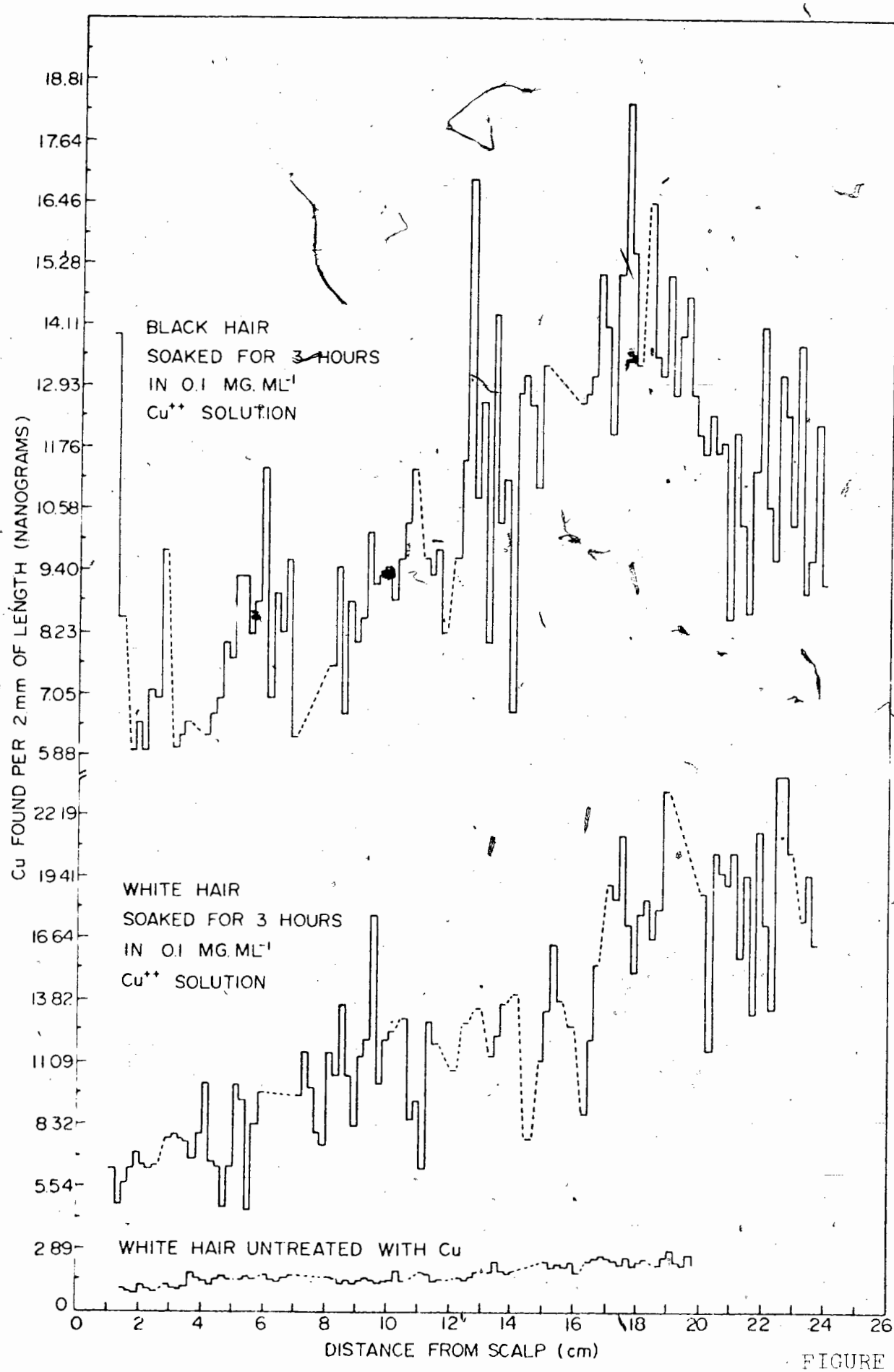

BLACK HAIR
SOAKED FOR 3 HOURS
IN 0.1 MG. ML⁻¹
Cu⁺⁺ SOLUTION
WHITE HAIR
SOAKED FOR 3 HOURS
IN 0.1 MG. ML⁻¹
Cu⁺⁺ SOLUTION
WHITE HAIR UNTREATED WITH Cu
Cu FOUND PER 2 mm OF LENGTH (NANOGRAMS)
18.81
17.64
16.46
15.28
14.11
12.93
11.76
10.58
9.40
8.23
7.05
5.88
22.19
19.41
16.64
13.82
11.09
8.32
5.54
2.89
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DISTANCE FROM SCALP (cm)

FIGURE 10

It is therefore reasonable to assume that the increase with distance from the root in the natural copper content may in large part, or entirely, be due to an increasing capacity for absorption (or decreasing inhibition of absorption) of copper from the environment with distance from the root, rather than to the increased exposure time, as previously speculated. This variation in the absorption capacity along the hair shaft is presumably linked to changes in hair structure (due e.g. to protein oxidation) during maturation or exposure to the atmosphere.

In addition, in the case of subject A, where the indigenous copper concentration was found to go through a maximum at particular sites along the hair (Fig. 6), it can be seen that the amount of copper taken up during soaking of hairs from the same subject and plucked at the same time is maximum at similar locations along the hair. This indicates that such regions of increased copper concentration are not just indicative of a dietary Cu fluctuation, but may be partly or completely the result of a variation in the absorption capacity of the hair for copper at particular sites along the hair which may be specific for a given individual.

3.1.5.2) Radioactive Tracer

The use of radioactive copper solutions for a soaking experiment seemed an interesting opportunity to verify the somewhat surprising

lts of the previous section, but by an alternative measurement technique.

In Fig. 11 data are shown on four hairs taken from subject A. In the top part of the figure are shown the patterns in two hairs plucked at the same time for indigenous copper and for copper added as inactive tracer: these data were drawn from Fig. 2 and 9. The bottom part of Fig. 11 shows the patterns obtained for two hairs plucked at a date 4 months later and treated with radioactive copper tracer. In each case a region of increased copper concentration is found, for the first two hairs, at about 6 cm from the root, and in the case of the latter two hairs, at about 10 cm. This difference in the position of the region of increased copper uptake is consistent with the length of hair which would have grown during the 4 month period separating the dates of acquisition of the two pairs of hairs, given the average growth rate quoted in the literature (0.35 mm/day) (Fle. 54).

This result confirms the above conclusions regarding the existence of regions of different absorption capacity for copper along the hair shaft. Microscopic examination using a scanning electron microscope failed, however, to reveal any morphological features which could be correlated with such regions.

This series of experiments was extended to hairs from subject E because of very particular features which were observed during the

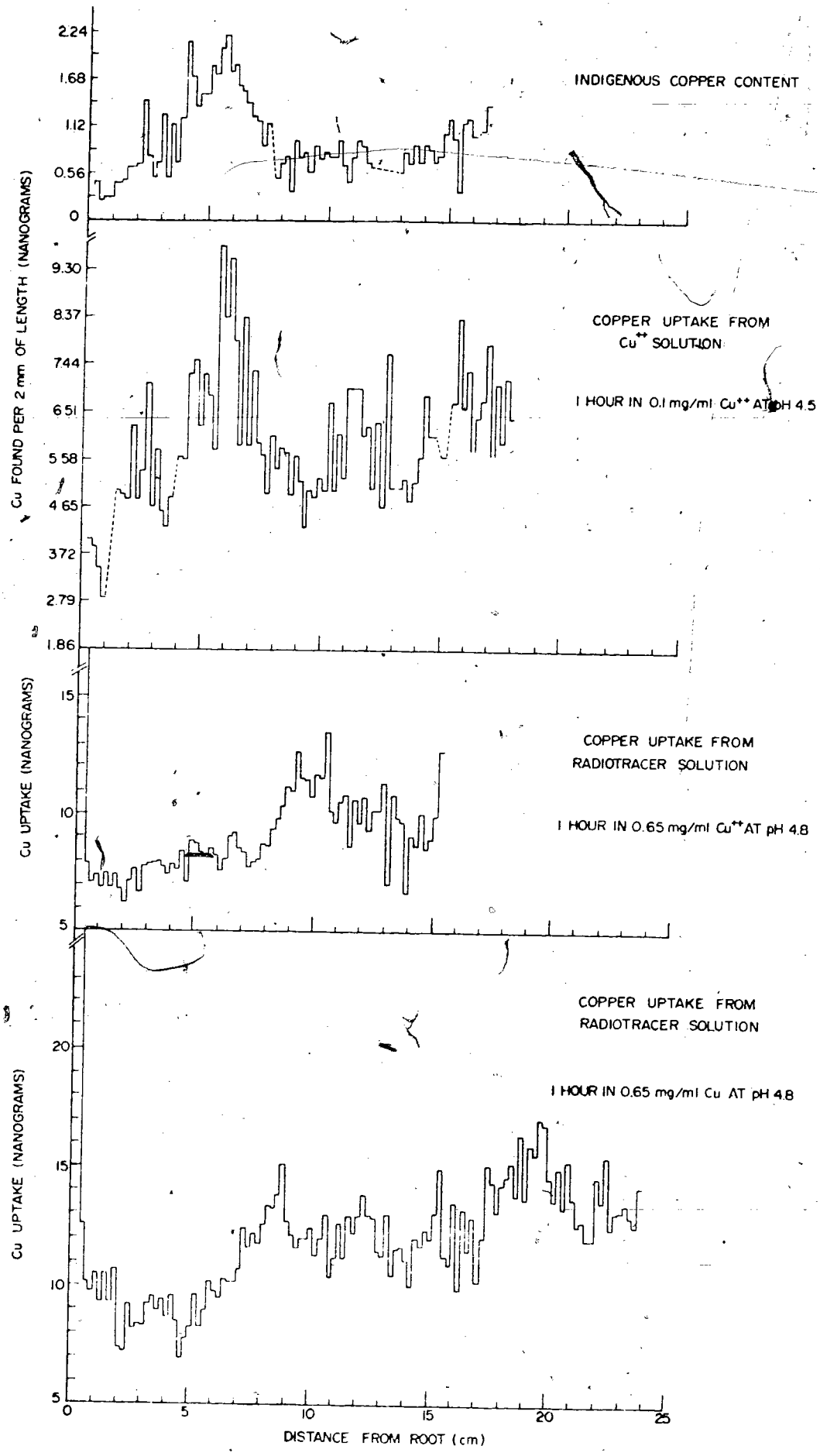

INDIGENOUS COPPER CONTENT
COPPER UPTAKE FROM
Cu++ SOLUTION
1 HOUR IN 0.1 mg/ml Cu++ AT pH 4.5
COPPER UPTAKE FROM
RADIOTRACER SOLUTION
1 HOUR IN 0.65 mg/ml Cu++ AT pH 4.8
COPPER UPTAKE FROM
RADIOTRACER SOLUTION
1 HOUR IN 0.65 mg/ml Cu AT pH 4.8
Cu FOUND PER 2 mm OF LENGTH (NANOGRAMS)
Cu UPTAKE (NANOGRAMS)
Cu UPTAKE (NANOGRAMS)
DISTANCE FROM ROOT (cm)

FIGURE 11

experiment on copper ingestion, discussed later. It was observed particularly that sometimes, shortly after shampooing, some of the hairs taken from this subject did not then exhibit the concentration pattern for indigenous copper shown for this subject in Fig. 2, but instead showed a rather constant natural copper content along the hair. This suggested a possible partial washing out of the natural copper from the distal part of the hair, a phenomenon not observed with any other subject examined.

The results of a radio-tracer experiment shown in Fig. 14, for five hairs, which were taken from subject E over a period of 1 month, seem inconsistent with the natural copper content patterns of Fig. 2 .

However, they do correspond with the patterns of hairs taken shortly after shampooing (Fig. 16 and 20), which suggests that, for this subject at least, some part of the copper content is not as strongly bound to the hair structure as for other subjects, and as such can be easily removed from the keratin either by shampooing, in the case of the natural copper, or by rinsing following the soaking in the radiotracer solution.

3.1.6) Effects of Hair Treatment

Two subjects involved in this study, subjects G and H, have used a bleaching agent on their hair, resulting in bleached and unbleached

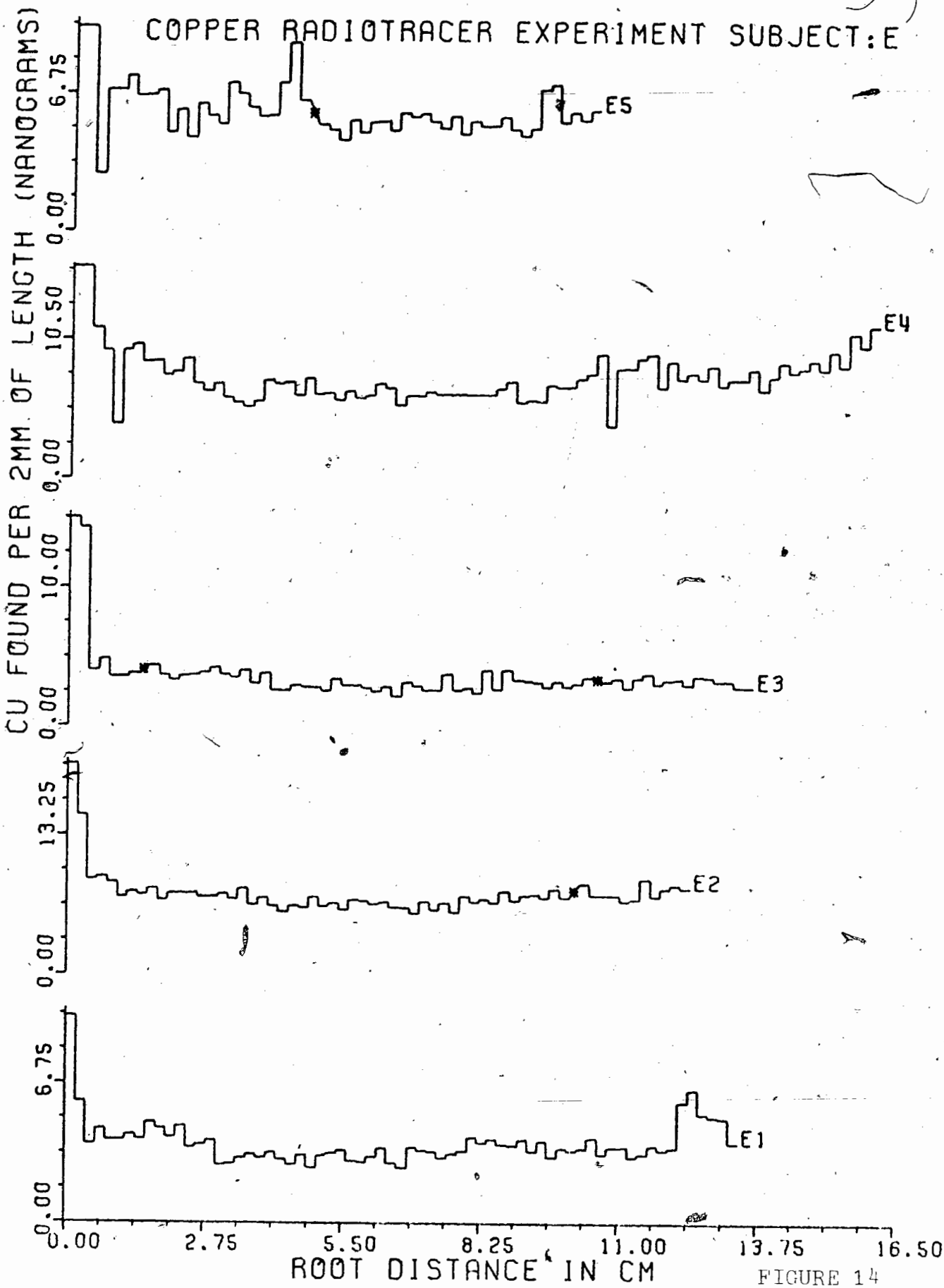

COPPER RADIOTRACER EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
E5
E4
E3
E2
E1
0.00
6.75
10.50
10.00
13.25
2.75
5.50
8.25
11.00
13.75
16.50
ROOT DISTANCE IN CM

FIGURE 14

regions with a relatively sharp colour change between the two. the results of indigenous copper measurements are shown in the left part of Fig. 13 for subject G (performed in an undergraduate exercise by the subject concerned), and in the lower part of Fig. 2 for subject H.

It is seen that, in both cases, the region of the hair treated with the bleaching solution exhibits an increased indigenous copper concentration. In addition to the bleaching treatment, for subject H the hairs were redyed shortly after the experiment so that no sharp colour change was then visible along the hair shaft. It seemed interesting to see if, in such a case, the region which used to exhibit an increased copper concentration (at approximately 13.0 cm from the root to the distal end) would still do so. Two such hairs (samples H_2 and H_3) were analyzed, and the resulting patterns are shown in Fig. 2g. It is seen that for both samples, the previously bleached region no longer showed the strongly increased Cu concentration. Thus, the dye employed evidently made less available the sites in the bleached hair structure to which copper had previously been bound. The importance of the observed phenomenon in forensic applications of the present analysis technique is obvious.

Further experiments were conducted with one hair drawn from subject G, who had used a bleaching agent, resulting in a very characteristic pattern for the natural copper content we discussed previously (shown in Fig. 13). This hair was subjected to the ^{64}Cu

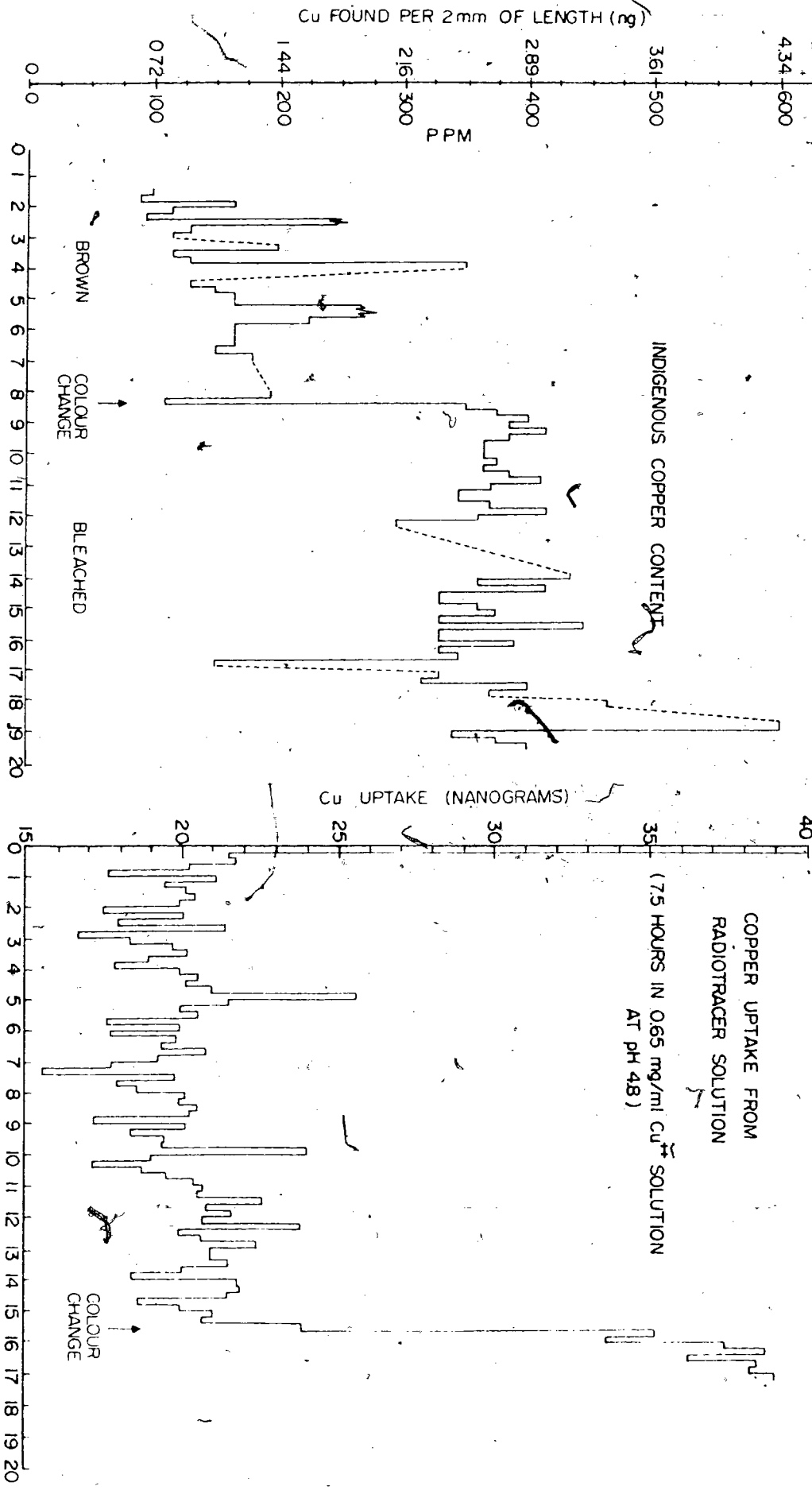

Cu FOUND PER 2mm OF LENGTH (ng)
0 10
0.72 100
1.44 200
2.16 300
2.89 400
3.61 500
4.34 600
PPM
INDIGENOUS COPPER CONTENT
BROWN
COLOUR CHANGE
BLEACHED
DISTANCE FROM ROOT (cm)
Cu UPTAKE (NANOGRAMS)
15 20 25 30 35 40
COPPER UPTAKE FROM RADIOTRACER SOLUTION
(7.5 HOURS IN 0.65 mg/ml Cu++ SOLUTION AT pH 4.8)
COLOUR CHANGE
DISTANCE FROM ROOT (cm)

FIGURE 15

radiotracer treatment, and the resulting variation of copper uptake from the radiotracer solution is shown on the right side of Fig. 13. It can be seen that the region of the hair treated with the bleaching solution exhibits an increased capacity to take up copper from radiotracer solution, producing a pattern similar to that for the natural copper content variation in the hair from the same subject.

In view of this result, it seemed interesting to see if the hairs of subject H, which were dyed after having been partly bleached, would show a constant uptake of radioactive copper along the hair shaft corresponding to the flat pattern for the natual copper content, as discussed previously. The result obtained is shown in Fig. 12, and clearly demonstrates the presence of an increase in the copper uptake in the region where the hairs were previously bleached (although the variation of copper uptake is not so important as in the case of subject G, whose hairs were not subjected to dyeing). This result suggests that the soaking of radioactive copper into hair can be used to reveal the existence of natural as well as artificial regions of increased or decreased copper absorptivity, even after a dyeing treatment has made impossible the visual recognition of, for example, a corresponding artificial colour change. Therefore, similarities of the radio-copper uptake patterns could be, under certain circumstances, a better means of identification than the natural copper content pattern already indicated to be specific of a given individual.

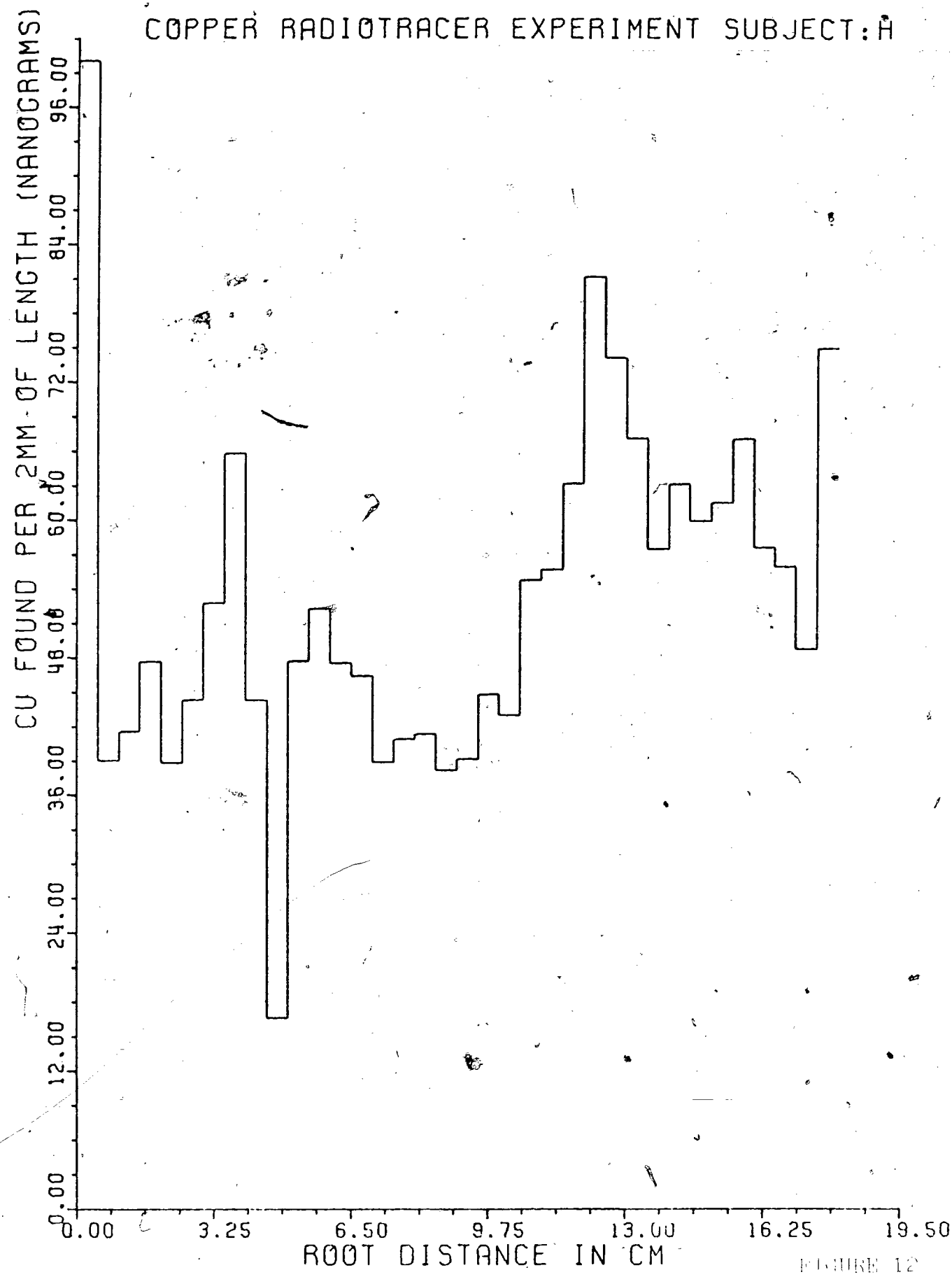

COPPER RADIOTRACER EXPERIMENT SUBJECT:A
CU FOUND PER 2MM-OF LENGTH (NANOGRAMS)
0.00
12.00
24.00
36.00
48.00
60.00
72.00
84.00
96.00
0.00
3.25
6.50
9.75
13.00
16.25
19.50
ROOT DISTANCE IN CM

FIGURE 12

3.1.7 Ingestion Experiments

The results of the foregoing experiments, namely that the patterns for natural copper content and those for copper added from solution were very similar, were consistent with the interpretation that the natural copper content of hair arises entirely from external sources such as sweat or environmental contamination.

It seemed desirable, therefore, to determine whether ANY features of the indigenous Cu pattern arose from copper incorporated via the follicle as the hair grows.

Analysis of hairs from two subjects, whose diets were modified by ingestion of elevated quantities of copper at known times, was expected to illuminate this question. The experimental techniques employed were described in section 2.6.

The results of the blood serum analysis for copper, undertaken simultaneously with the hair analysis, are given in Table VIII, together with the time at which the blood was taken.

It can be seen that very little correlation exists between the blood copper concentration and the time of the ingestion. It seems, furthermore, that results obtained by the X-Ray Fluorescence Technique are consistently lower than the values given by the Atomic Absorption

TABLE VIII

Copper concentration in blood serum

Time of Sampling	Cu Concentration g/100ml Serum	
	XRF	AA
Subject E		
2 days before ingestion	142	176
1 day before ingestion	160	90
INGESTION		
1 day after ingestion	95	112
3 days after ingestion	78	116
12 days after ingestion	88	112
Subject F		
9 days before ingestion	75	82
5 days before ingestion	85	126
1 day before ingestion	116	140
INGESTION		
2 days after ingestion	170	134
5 days after ingestion	153	168
21 days after ingestion	145	126

Analysis. Thus it is difficult to draw any conclusions on the influence of the copper ingestion on the concentration of this metal in blood serum.

In order to attempt detection of a possible change in the Cu pattern in the hair of subject F following ingestion, three samples taken from this subject prior to the Cu ingestion were first analysed followed by hairs taken at various times after the ingestion. The times at which other hair samples were taken from subject F are given in Table IX together with the number of hairs analysed in each case.

No significant increase of copper content was ever observed along any of the hairs analysed as long as 18 days after the copper ingestion. Especially, the copper content in the first 2 cm of the hairs remained at the same low level which was characteristic of samples taken from the subject the day before the ingestion. This absence of response of the hair copper level to the quantity of copper ingested by subject F was attributed to a possible insufficiency in the amount of copper administered. It was then decided to repeat the same experiment with another subject (E), who ingested 40 mg of copper instead of 20 mg. Blood analyses were again performed with inconclusive results, as shown in Table VIII.

Seven hair samples taken from this subject prior to ingestion were analysed as described in section 2.6. Three of these samples

TABLE IX

Times when hair and blood were taken during the copper ingestion experiment on Subject F

Time of Blood Sampling	Time of Hair Sampling	Number of Hairs Analysed
9 days before ingestion		
5 days before ingestion		
1 day before ingestion	1 day before ingestion	2
	DAY OF INGESTION	2
	1 day after ingestion	2
2 days after ingestion		
	4 days after ingestion	2
5 days after ingestion	5 days after ingestion	2
	14 days after ingestion	1
	18 days after ingestion	1
21 days after ingestion		

TABLE X

Times when hair and blood were taken during the copper ingestion experiment of Subject E

Time of Blood Sampling	Time of Hair Sampling	Number of Hairs Analyzed
2 days before ingestion		
1 day before ingestion	1 day before ingestion	7
	INGESTION	
1 day after ingestion	1 day after ingestion	3
	head shampoo	
3 days after ingestion	3 days after ingestion	2
	6 days after ingestion	4
	head shampoo	
	7 days after ingestion	2
	11 days after ingestion	3
	head shampoo	
12 days after ingestion	12 days after ingestion	2
	21 days after ingestion	2

have their natural copper content patterns reproduced in Fig. 2, and are characterized particularly by the low copper content (about 0.45 nanogram) of each 2-mm segment analysed between 0.4 cm and 2.6 cm from the root.

Results of the analysis for three hairs taken the day after the ingestion (Fig. 15) do not show any appreciable modification of the patterns compared to those for the samples taken up just before the ingestion, and the copper level in the region in the vicinity of the scalp remains close to 0.4 nanogram per 2-mm segment.

At successively 3 and 6 days after the ingestion (Fig. 16 and 17), a small increase of the copper level to 0.5 nanogram per 2-mm segment may be noticed in the same region of the hair shaft. At 7 days after the ingestion (Fig. 18) the copper content of each 2-mm segment has an average value of 0.78 nanogram for the two hairs analysed, which is significantly higher than the values measured for periods of time closer to the ingestion.

For 11, 12, and 21 days after the ingestion, the copper level in the region of the hair close to the root returns to its original value (0.4 nanogram per 2-mm segment). The patterns corresponding to hairs taken at these specific dates are shown respectively in Fig. 19, 20, and 21.

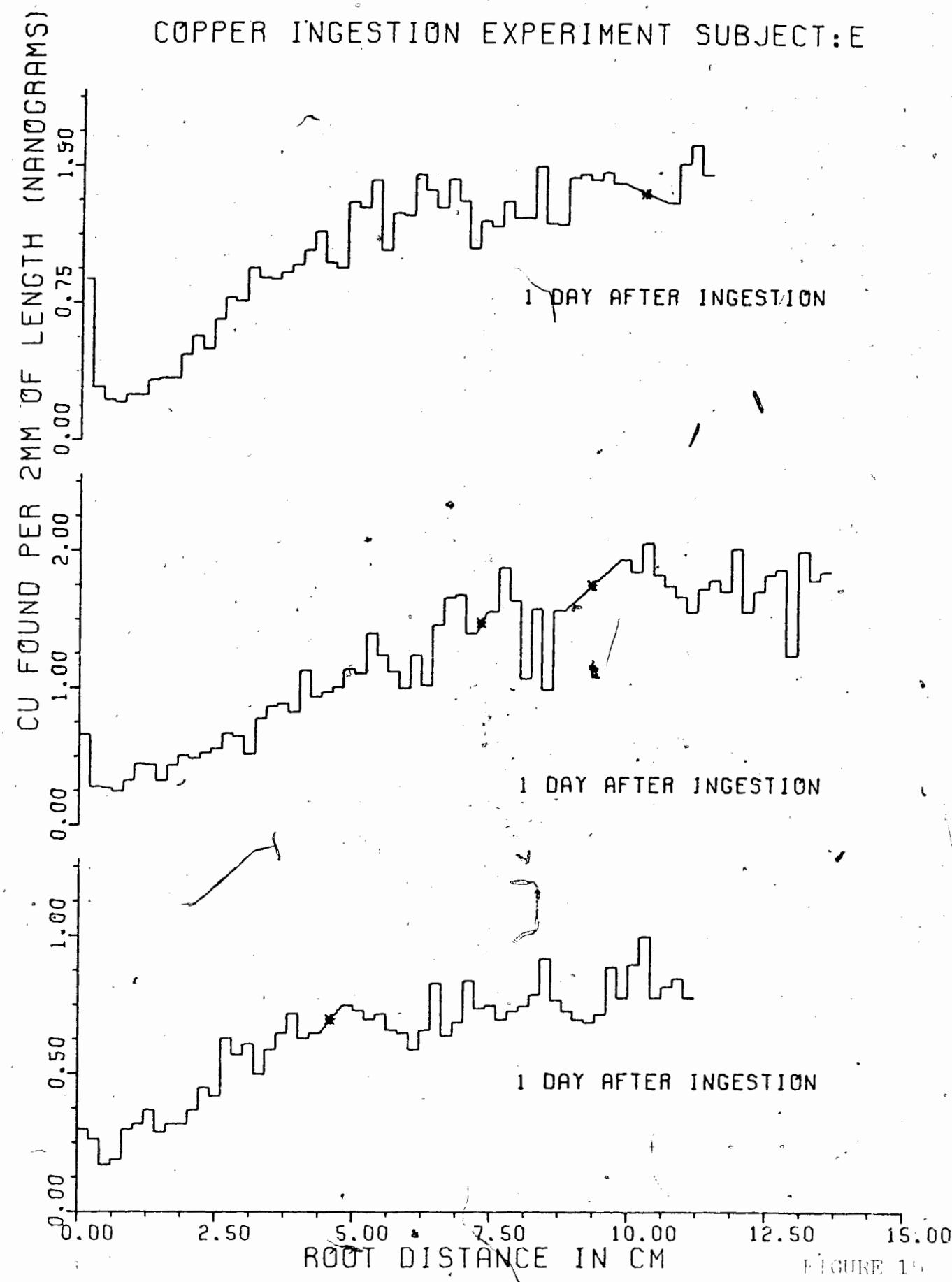

COPPER INGESTION EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
0.75
1.50
1 DAY AFTER INGESTION
0.00
1.00
2.00
1 DAY AFTER INGESTION
0.00
0.50
1.00
1 DAY AFTER INGESTION
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 1

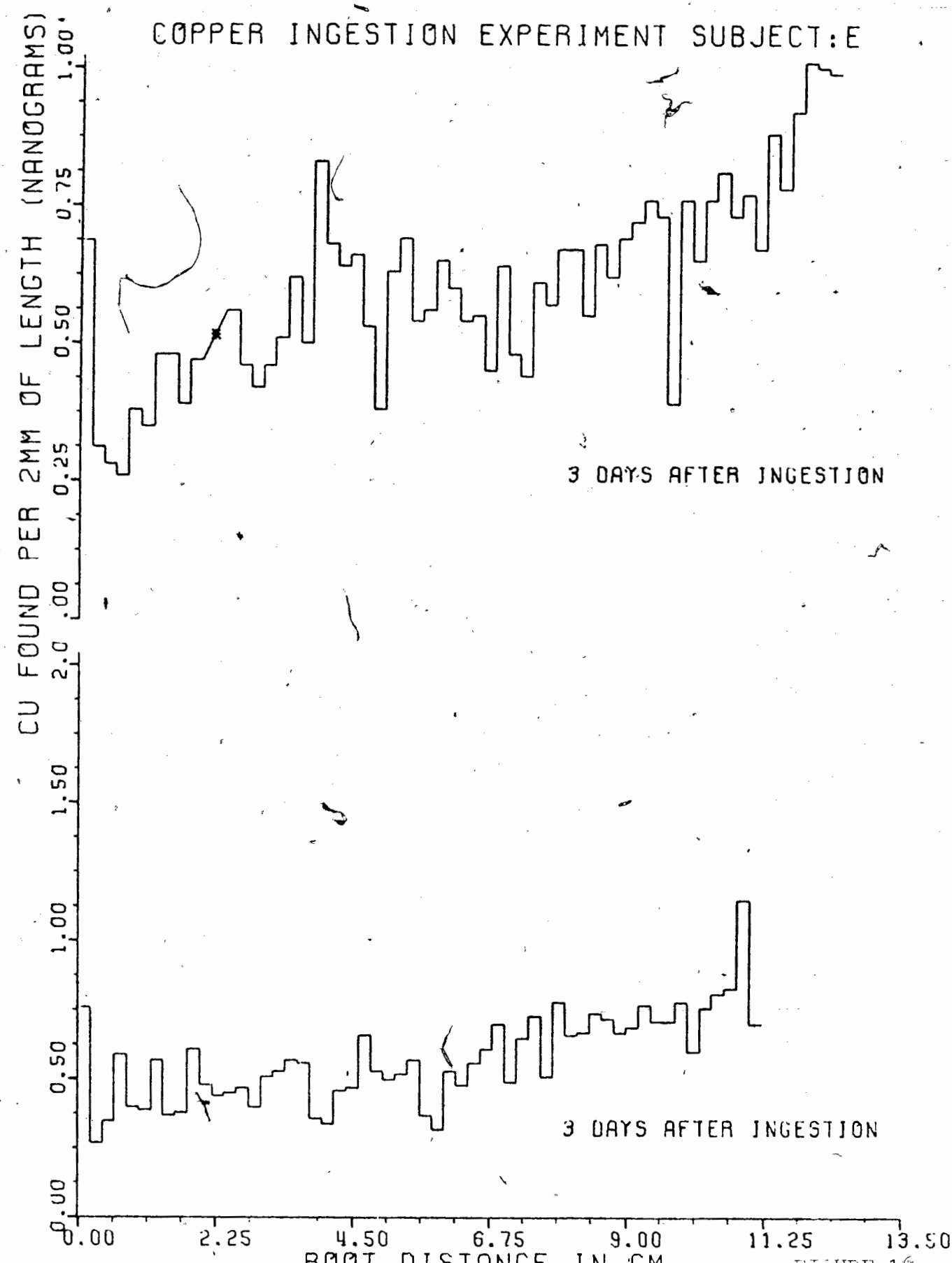

COPPER INGESTION EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
1.00
0.75
0.50
0.25
.00
3 DAYS AFTER INGESTION
2.0
1.50
1.00
0.50
0.00
3 DAYS AFTER INGESTION
0.00
2.25
4.50
6.75
9.00
11.25
13.50
ROOT DISTANCE IN CM

FIGURE 16

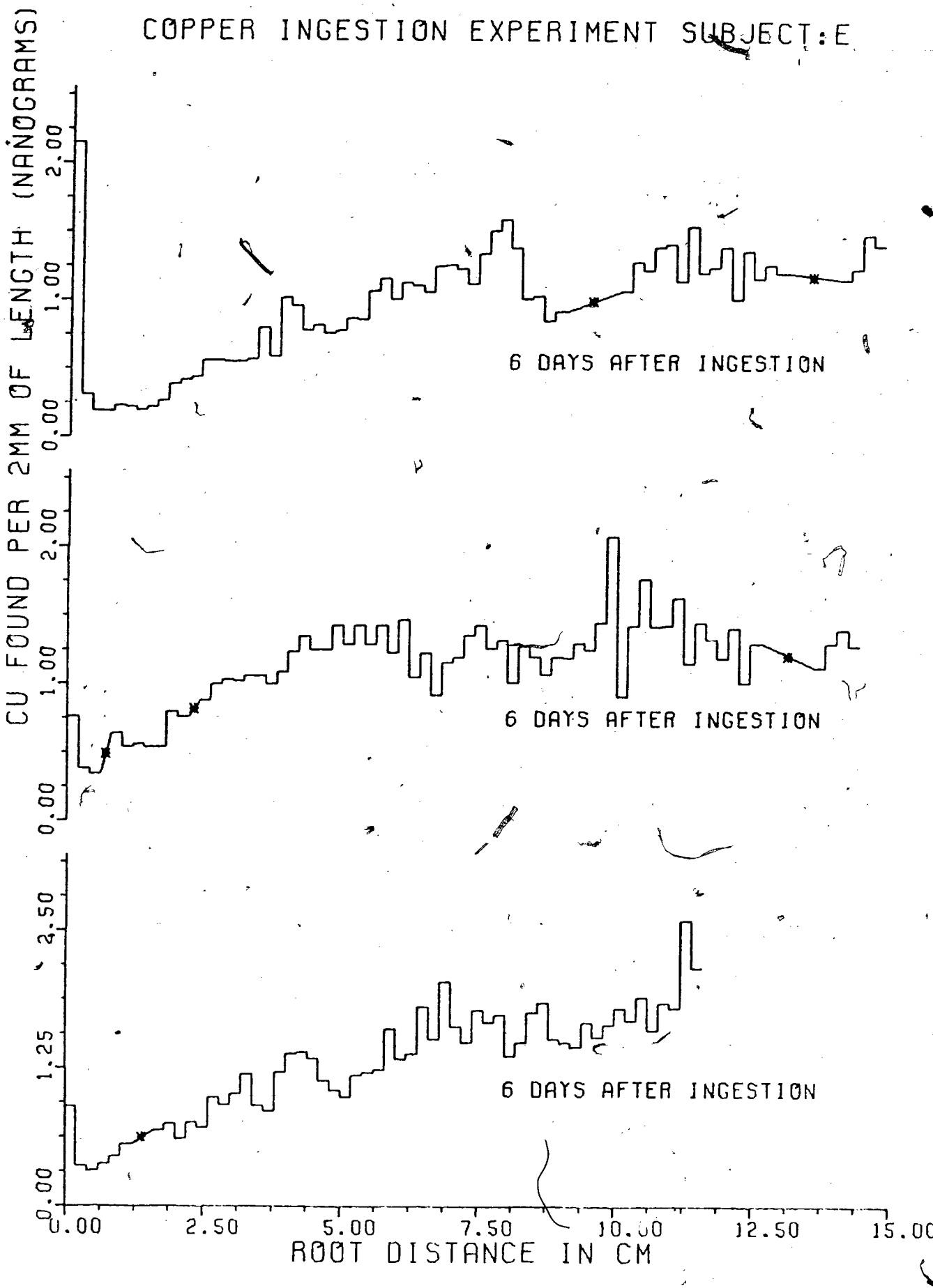

COPPER INGESTION EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
1.00
2.00
6 DAYS AFTER INGESTION
0.00
1.00
2.00
6 DAYS AFTER INGESTION
0.00
1.25
2.50
6 DAYS AFTER INGESTION
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 17

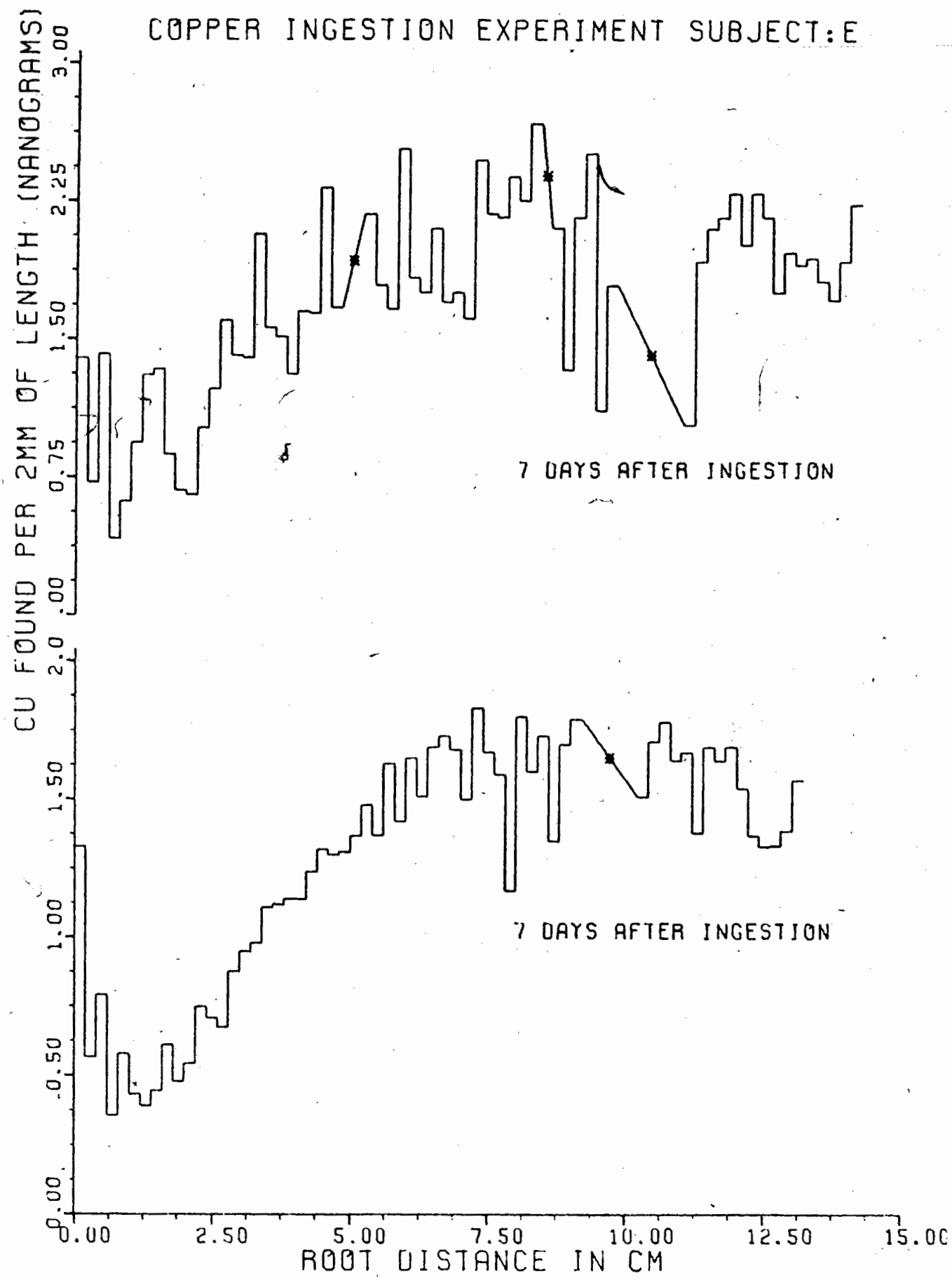

COPPER INGESTION EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
3.00
2.25
1.50
0.75
.00
7 DAYS AFTER INGESTION
2.0
1.50
1.00
.50
0.00
7 DAYS AFTER INGESTION
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 18

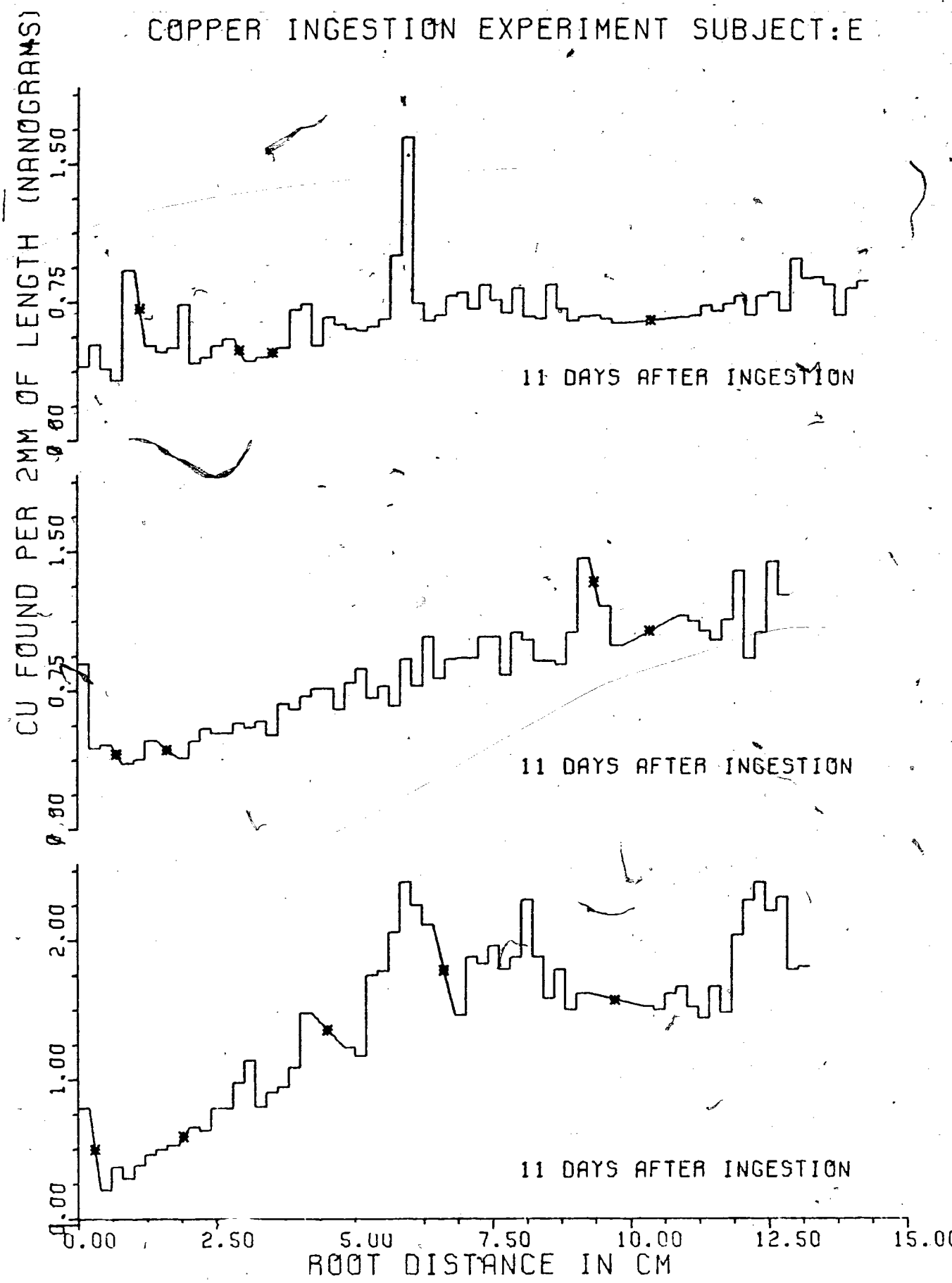

COPPER INGESTION EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
0.75
1.50
11 DAYS AFTER INGESTION
0.00
0.75
1.50
11 DAYS AFTER INGESTION
0.00
1.00
2.00
11 DAYS AFTER INGESTION
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 19

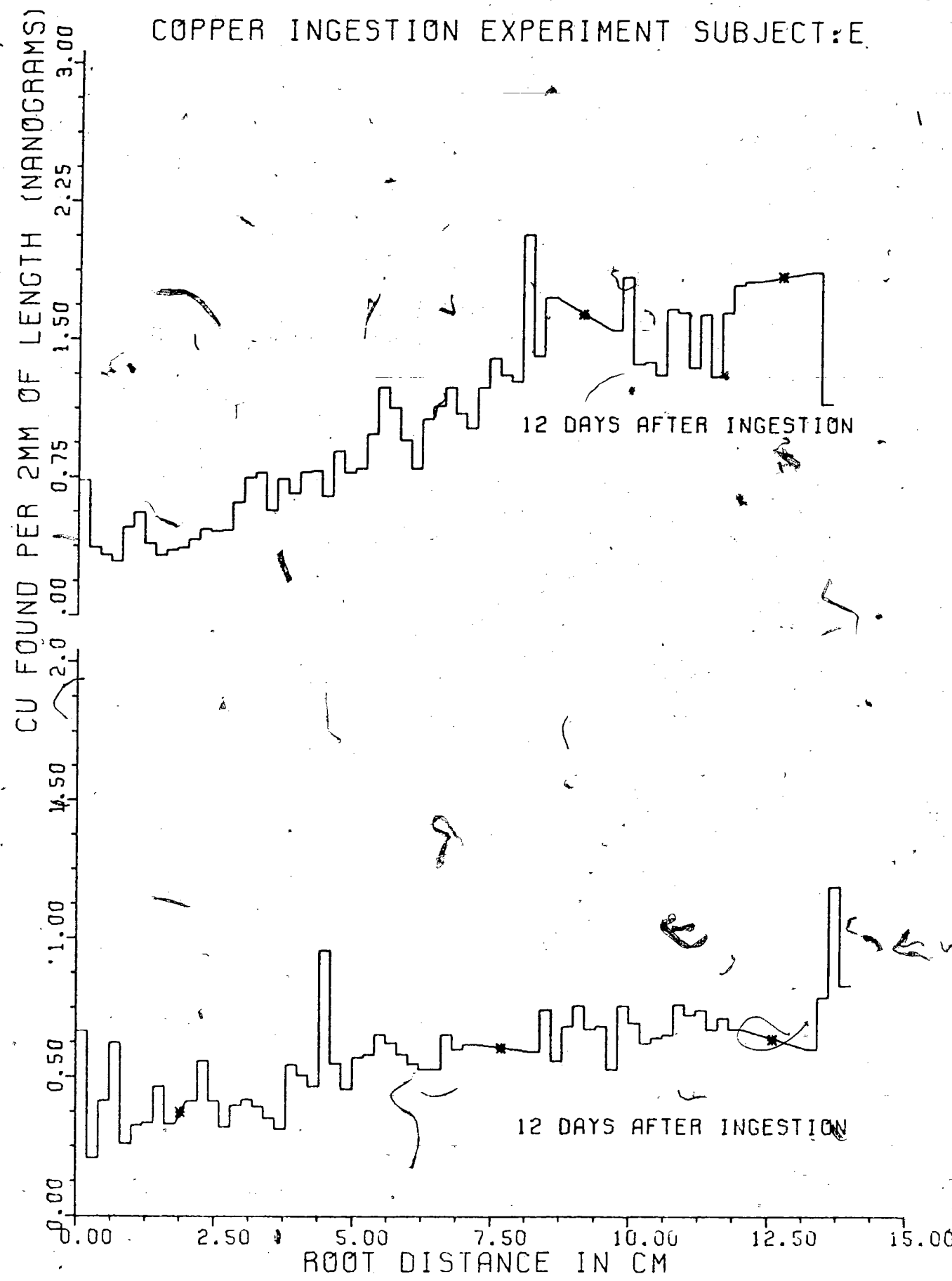

COPPER INGESTION EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
3.00
2.25
1.50
0.75
.00
12 DAYS AFTER INGESTION
2.0
1.50
1.00
0.50
0.00
12 DAYS AFTER INGESTION
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 20

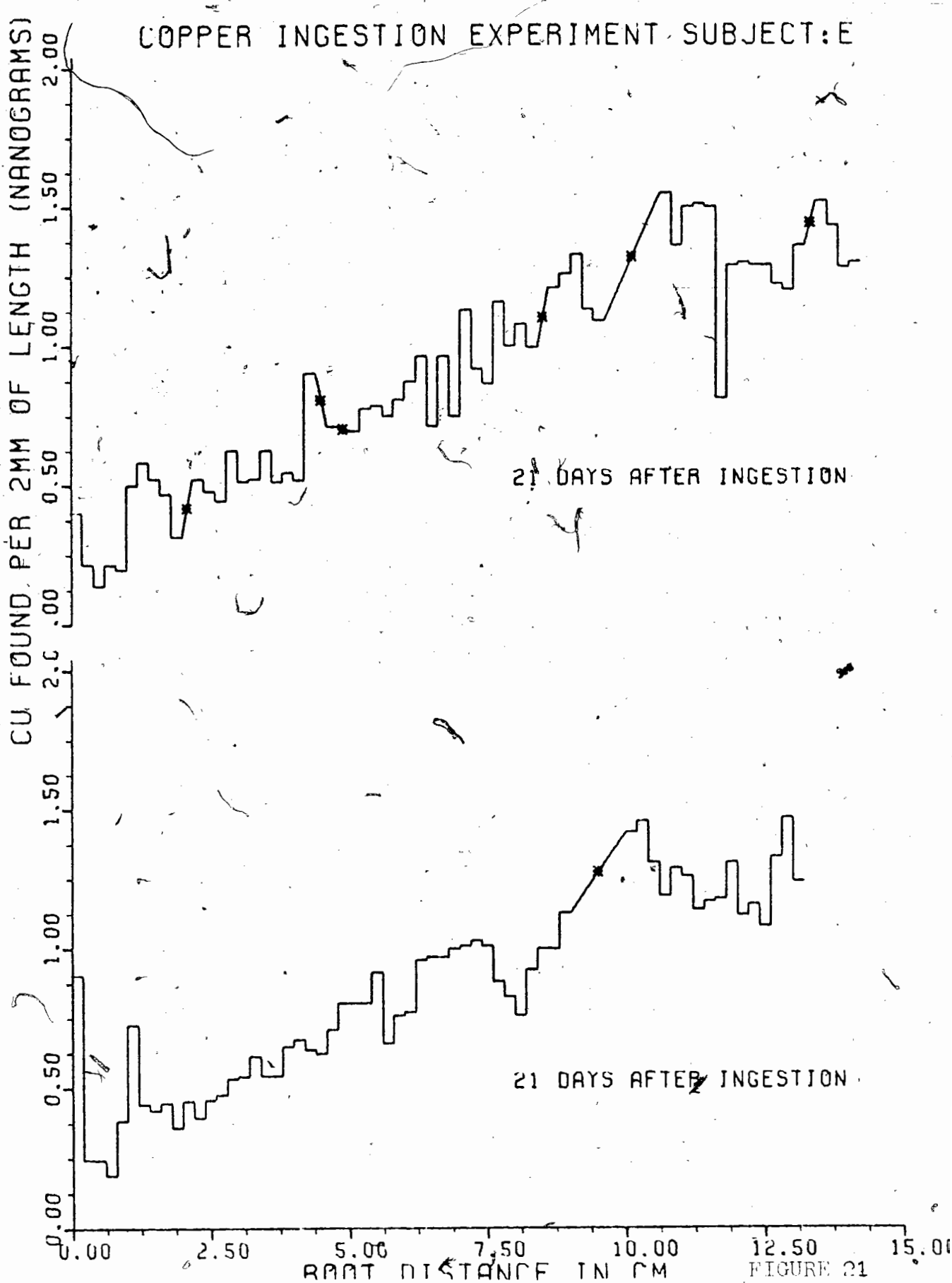

COPPER INGESTION EXPERIMENT SUBJECT:E
CU FOUND PER 2MM OF LENGTH (NANOGRAMS)
2.00
1.50
1.00
0.50
.00
21 DAYS AFTER INGESTION
2.0
1.50
1.00
0.50
0.00
21 DAYS AFTER INGESTION
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 21

In order to facilitate the interpretation of the results discussed in this section, the average copper content has been calculated for hair segments of various length in the immediate vicinity of the root. These values are given in Table XI.

It seems, therefore, that only hairs taken 7 days after the ingestion show an increase of copper level in the region of the hair extending from 0.4 to 2.6 cm, as well as in narrower zones such as 1.0 to 2.0 cm from the root. It seems important, then, to test if the copper level reached at this date is significantly different from the averaged value calculated on the seven hairs taken the day before the ingestion. The calculated values of the t-test show that these average copper contents of the hair extending from 0.4 to 2.6 cm or from 1.0 to 2.0 cm from the root, are different at the 96% level of confidence. It seems, then, that attribution of the presence of copper in this part of the hair to a mechanism which would involve even partially its incorporation into the keratin found in the follicle is unreasonable because:

- The high copper content region produced by this mechanism would probably not reach distances as far as 1.0 to 2.0 cm from the root in a 7 day period of hair growth.

- After 21 days, the region of the hair produced at the time of the ingestion (where the incorporation of copper would

TABLE XI

Average copper content (nanograms) for sections of various length along the hair fiber at different times after the ingestion

Date	Number of Hairs	Region of the Hair			
		0.4-2.6 cm	0.6-2.4 cm	0.8-2.2 cm	1.0-2.0 cm
1 day before ingestion	7	.473 ±.155	.447 ±.177	.432 ±.192	.405 ±.146
1 day after ingestion	3	.370 ±.062	.364 ±.062	.364 ±.068	.374 ±.064
3 days after ingestion	2	.441 ±.006	.445 ±.024	.655 ±.033	.449 ±.012
6 days after ingestion	4	.501 ±.152	.487 ±.148	.482 ±.148	.484 ±.155
7 days after ingestion	2	.786 ±.31	.719 ±.266	.73 ±.28	.725 ±.34
11 days after ingestion	3	.496 ±.04	.495 ±.052	.495 ±.079	.518 ±.016
12 days after ingestion	2	.391 ±.016	.408 ±.003	.404 ±.02	.404 ±.003
21 days after ingestion	2	.41 ±.009	.425 ±.004	.449 ±.006	.477 ±.003

have happened) should have grown to a distance of 7.5 mm from the root, where no maximum in the copper content has been detected in this position on either hair taken at that time after the ingestion.

It is therefore reasonable to assume that the copper which appeared on the hair shaft 7 days after the ingestion may have been transported there via the sweat, although such a mechanism would be expected to respond to the ingestion of high quantities of copper in a much shorter time.

Independently of the results previously described, some of the patterns obtained in the course of this experiment were dissimilar to the curves which were characteristic of subject E prior to ingestion (Fig. 2). In particular, 3, 11, and 12 days after the ingestion, some of the hairs taken from this subject showed a rather constant natural copper content all along the hair, which is reminiscent of the behaviour observed during the experiment with radioactive tracer (section 3.1.5.2) with the hairs from the same subject.

Because two of the three sets of hair which displayed such a feature were taken shortly after the subject had washed his head, it is tempting to assume a partial washing out of the natural copper from the distal part of the hair by the detergent employed. However, this does not explain why such a flat pattern has not been found for hairs

taken 7 days after the ingestion, since the subject washed his head again shortly before that time, and why, 11 days after the ingestion, one of the hairs analysed showed no increase of copper content along its length although no shampooing had occurred in the preceeding 3 days.

It seems, then, that a marked inconsistency prevails for the results obtained with the hairs of subject E, although it must be noted that all the hairs taken prior to ingestion showed a similar concentration pattern (Fig. 2) (with a correlation coefficient of 0.914). Likewise, soaking of copper into hairs taken from the day before the ingestion to the 21st day after the ingestion (Fig. 14) produced patterns consistent among themselves and similar to the concentration patterns of hairs of which the natural content may have been washed out by shampooing.

In conclusion, it seems impossible to disregard completely the possibility for at least a fraction of the copper observed in hairs to originate from incorporation via the root through the growth process. However, a larger amount of this metal would probably have to be ingested by the subject to clearly reveal this mechanism, a procedure which is evidently impossible for medical reasons.

3.2) Experiments on Zinc

Some similarity between concentration patterns for zinc and

copper was to be expected, perhaps since both are essential elements to animal biochemistry, and in addition both exist in solution as hydrated divalent ions.

However, zinc was reported to be the only element whose natural concentration does not vary appreciably along the hair length (Obr.#72), and unlike copper, which seems to be strongly bonded to the hair structure, zinc has been shown to be easily extractable from the keratin (Hin.#74).

3.2.1)#Results for Indigenous Zinc in Hair

Because of the high concentration of zinc in hairs, and the remarkable sensitivity of Atomic Absorption Analysis for this element, it was not possible to analyse the usual 2-mm segments, but instead smaller segments of 1.0- or 0.5-mm length had to be used, which is the minimum size that can be handled by the technique described in section 2.3.

Preliminary analysis of a few hairs, however, indicated that large segment-to-segment variation in the zinc content could hide some relevant features of the concentration pattern. It was therefore decided to increase the length of the washing time from 5 to 10 minutes per step of the cleaning procedure previously employed for the analysis of copper, and described in section 2.2.

The validity of the measurements of the natural zinc content of hair was checked from time to time by adding into the furnace 10 µl of ultra pure HNO_3 after the sample was ashed. Then, the drying and ashing processes were restarted and followed by the usual atomization and measurement. Under such conditions, the zinc is expected to dissolve in the nitric acid during the "drying" step (which lasted 30 seconds), and hence the residue to have the same chemical form and environment as in the case of calibration with an aliquot of a standard solution, which was made up from zinc metal dissolved in nitric acid.

It was found that after such treatment, the amount of zinc measured was similar to the value obtained via normal analysis of a hair sample. A perfect agreement is, of course, not expected, due to the important segment-to-segment concentration variation observed for this metal.

Two hairs taken from the head of subject B were analysed for their natural zinc content: the patterns obtained are shown in Fig. 22, where no increase in the zinc concentration can be observed along the length of the hair.

This result is similar to the conclusion of Obrusnik et al. (Obr. 72) (for hairs from subject A) obtained via Neutron Activation

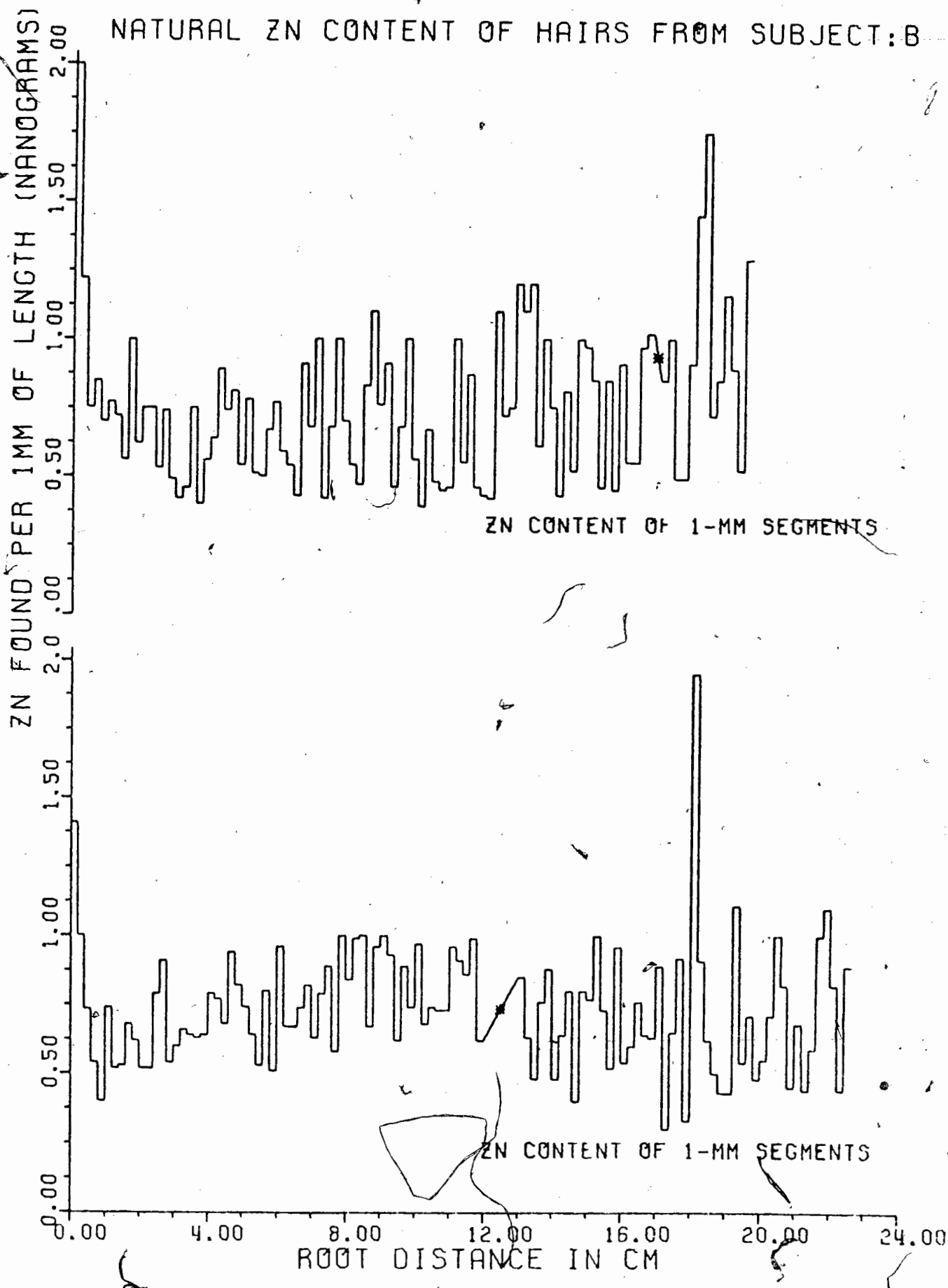

NATURAL ZN CONTENT OF HAIRS FROM SUBJECT:B
ZN FOUND PER 1MM OF LENGTH (NANOGRAMS)
.00
0.50
1.00
1.50
2.00
ZN CONTENT OF 1-MM SEGMENTS
0.00
0.50
1.00
1.50
2.0
ZN CONTENT OF 1-MM SEGMENTS
0.00
4.00
8.00
12.00
16.00
20.00
24.00
ROOT DISTANCE IN CM

FIGURE 22

Analysis of successive 10 cm of the hair.

These two patterns can be contrasted to the copper concentration patterns (shown in Fig. 2), obtained for similar hairs taken from the same subject, which were characterized by a large increase of the natural Cu content along the hair length.

Similarly, two hairs from subject I were analysed by Atomic Absorption Spectroscopy for zinc concentration. It can be seen again in Fig. 23 that the Zn content of each 1-mm (or 0.5-mm) segment analysed remains approximately constant along the whole hair length.

It is possible to interpret these results in three different ways:

The flat patterns obtained for these two subjects may suggest we are looking at the result of the incorporation of zinc into the hairs through the follicle, as the hairs grew at roughly a constant rate.

- The absence of zones of preferential absorption for zinc (such as the ones found for Cu) may have resulted in a constant uptake of zinc from external contamination (including sweat and fallout) along the entire hair length.

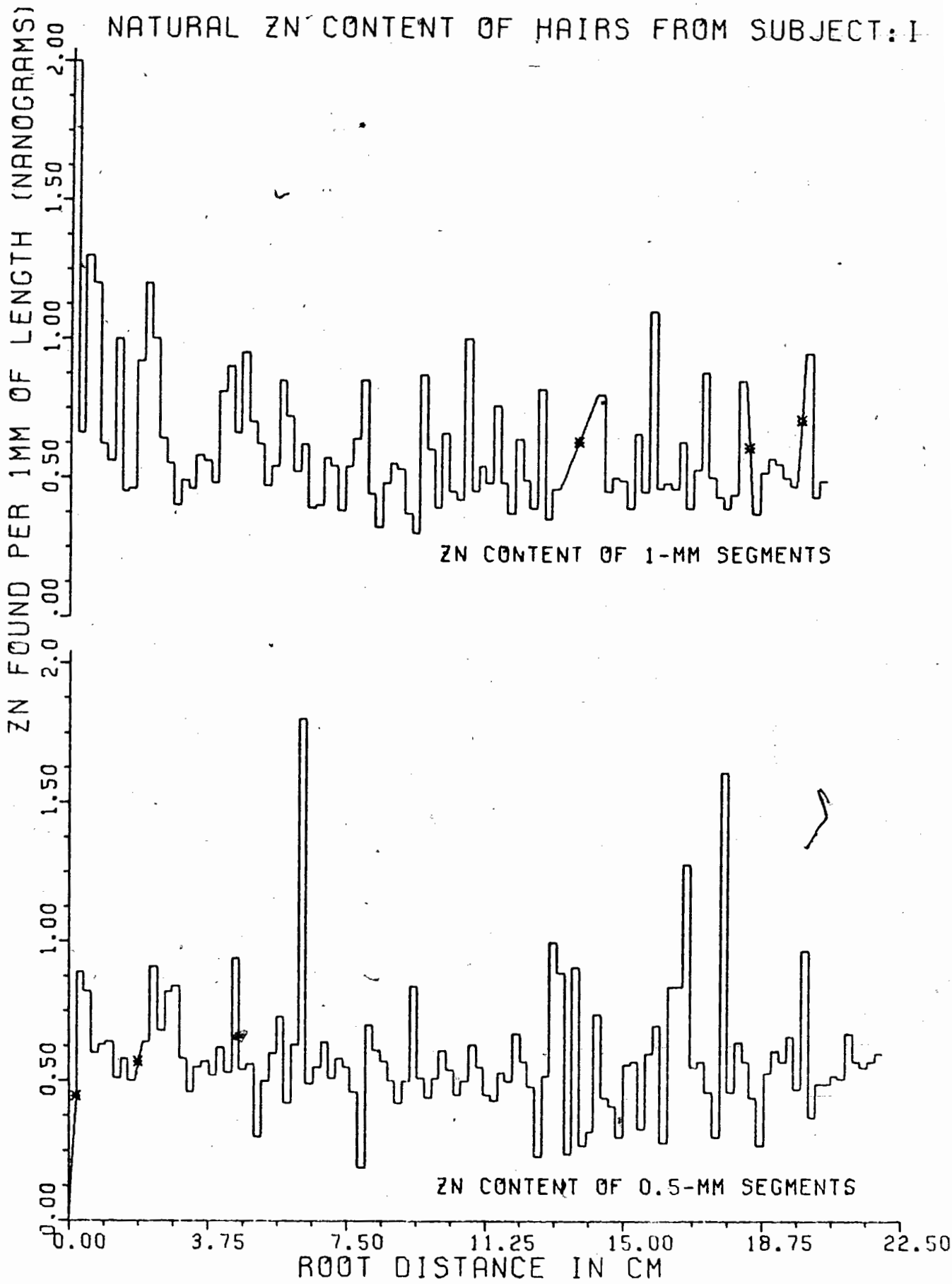

NATURAL ZN CONTENT OF HAIRS FROM SUBJECT: I
ZN FOUND PER 1MM OF LENGTH (NANOGRAMS)
2.00
1.50
1.00
0.50
.00
ZN CONTENT OF 1-MM SEGMENTS
2.0
1.50
1.00
0.50
0.00
ZN CONTENT OF 0.5-MM SEGMENTS
0.00
3.75
7.50
11.25
15.00
18.75
22.50
ROOT DISTANCE IN CM

FIGURE 23

- The zinc, which may have been absorbed preferentially in certain regions of the hair (i.e. the more distal part of the shaft), is more intensively washed out from those regions than from any other zone of the hair by the rather long washing procedure the samples were submitted to (90 minutes).

However, the results obtained so far do not present sufficient evidence to reject any of these hypotheses. It was therefore decided to examine the absorption of zinc in hair by means of a tracer experiment.

3.2.2) Tracer Experiment

Two hairs plucked from the head of subject B were subjected to a soaking in a solution of ^{65}Zn radioactivity with a concentration of 0.1 mg/ml and 3.6 mci/ml, the pH of which was adjusted to 5.5. The results are shown in Fig. 24, together with the length of time each hair was soaked in the solution.

A general increase of concentration with distance from the root, together with local regions of increased or decreased zinc uptake, are observed. These patterns are reminiscent of those observed in the case of both added and natural copper, which suggests these results could be interpreted once more in terms of a variation in absorption capacity of the hair structure for the zinc ions.

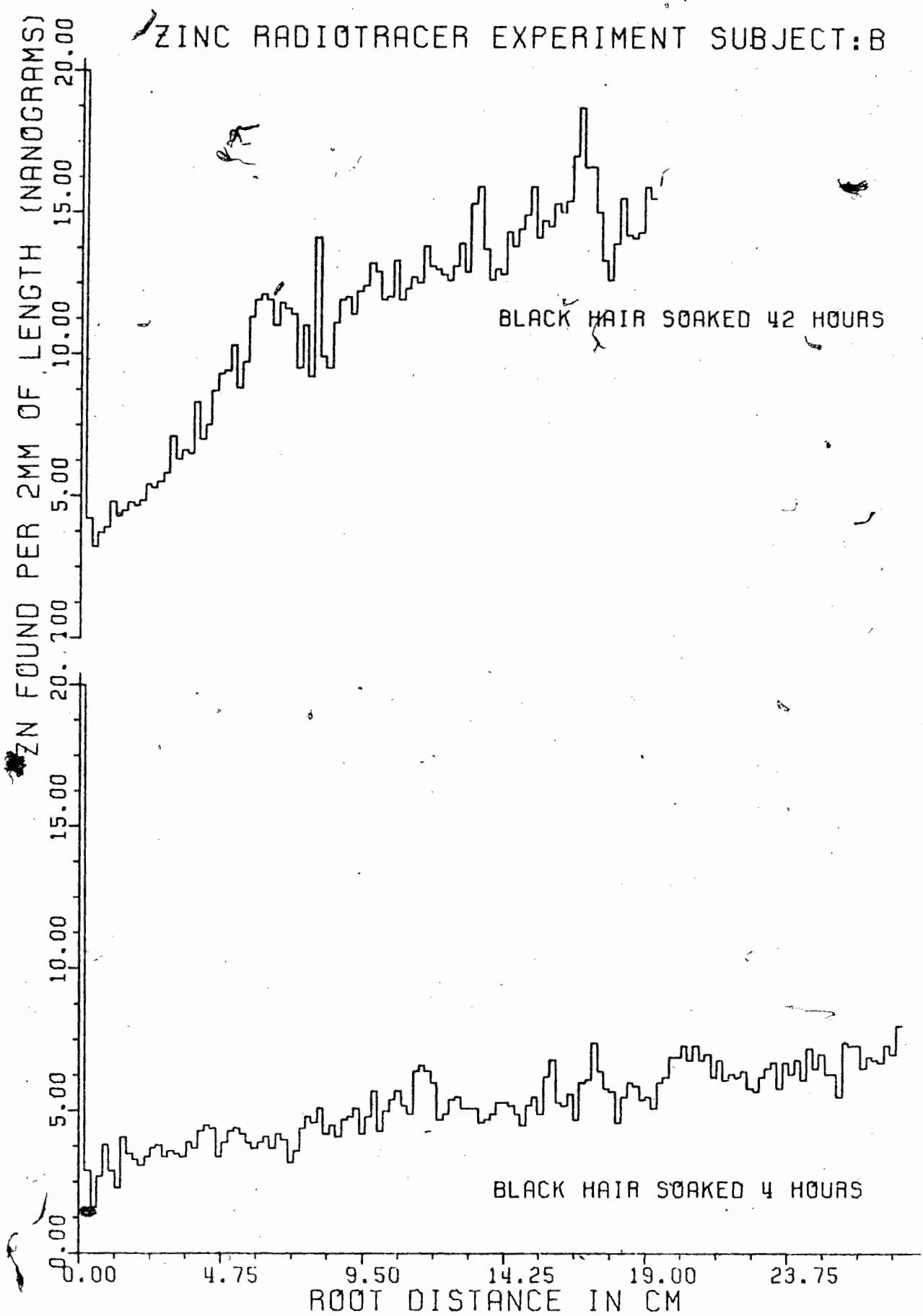

ZINC RADIOTRACER EXPERIMENT SUBJECT:B
ZN FOUND PER 2MM OF LENGTH (NANOGRAMS)
20.00
15.00
10.00
5.00
.100
BLACK HAIR SOAKED 42 HOURS
20.
15.00
10.00
5.00
0.00
BLACK HAIR SOAKED 4 HOURS
0.00
4.75
9.50
14.25
19.00
23.75
ROOT DISTANCE IN CM

FIGURE 24

However, the important dissimilarity observed between the patterns of natural and added zinc for the hairs of this subject (B), may indicate that the natural zinc may not be carried into the hair structure by the same mechanisms described previously for copper; the regions of increased zinc absorption of which the existence was revealed by the tracer experiment evidently did not produce any fluctuation in the natural concentration of this metal along the hair. Therefore, the zinc may originate entirely from incorporation via the follicle during hair growth, or may represent a residue from a washing out process.

The amount of zinc absorbed by each 2-mm segment is seen to be twice as large for the hair soaked for 42 hours as for the sample which remained in the solution for only 4 hours. The rate of diffusion of zinc ions into hairs, as it may be revealed by these data, is evidently much slower than the penetration of water into the same structure, which process has been shown to be complete in less than 1 hour (Ber. 67).

A similar experiment was repeated with the hairs of subject A, which were previously shown to possess a very characteristic pattern when analysed for the natural or added copper content.

However, because of the considerable change observed in the concentration patterns of subject B when the time of soaking in the

zinc solution was increased, it was decided now to maintain the soaking time constant to verify if a similarity of absorption patterns would be observed between the hairs taken from a single individual.

It seems that some similarities exist between the concentration patterns shown in Fig. 25 for three hairs taken from subject A, especially around 2 and 10 cm from the root where the presence of zones of increased and decreased absorption can be observed.

It must be noticed, however, that:

- The correlation existing between these three patterns is perhaps less obvious than in the case of added copper (Fig. 11) for the hairs of the same subject.

- These patterns, and the one shown for natural copper in Fig. 2, are much less correlated, although both sets of hairs were taken from subject A at the same time. That is to say, the zones of apparent absorption of Zn and of Cu are located at different distances from the root.

Similar conclusions are to be drawn from the data in Fig. 26 from two hairs from the same subject soaked for different lengths of time. It can be seen that the level of Zn absorbed in these hairs increased by a factor of 10 when the duration of the soaking time was increased

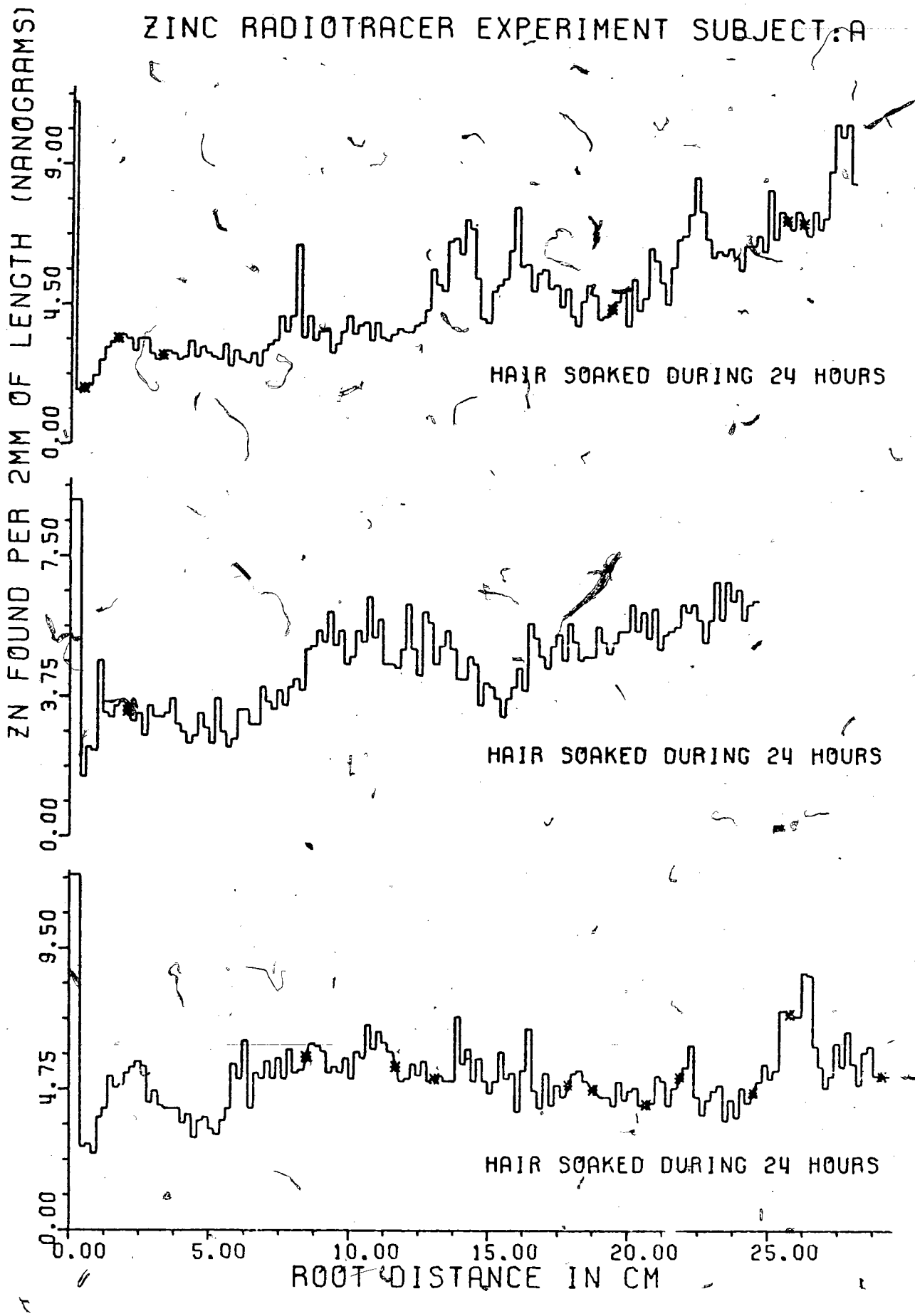

ZINC RADIOTRACER EXPERIMENT SUBJECT: A
ZN FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
4.50
9.00
HAIR SOAKED DURING 24 HOURS
0.00
3.75
7.50
HAIR SOAKED DURING 24 HOURS
0.00
4.75
9.50
HAIR SOAKED DURING 24 HOURS
0.00
5.00
10.00
15.00
20.00
25.00
ROOT DISTANCE IN CM

FIGURE 25

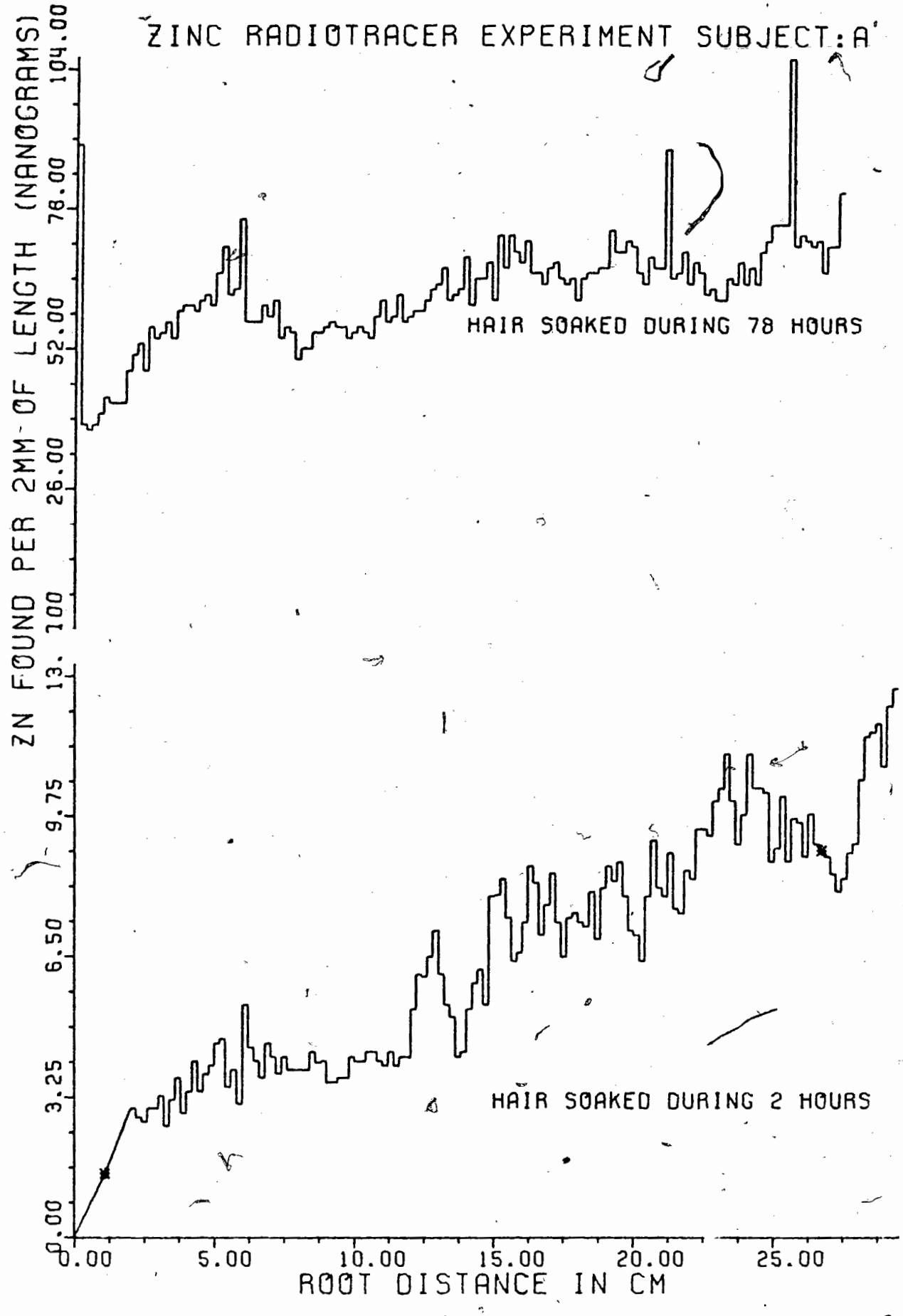

ZINC RADIOTRACER EXPERIMENT SUBJECT:A'
ZN FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
3.25
6.50
9.75
13. 100
26.00
52.00
78.00
104.00
HAIR SOAKED DURING 78 HOURS
HAIR SOAKED DURING 2 HOURS
0.00
5.00
10.00
15.00
20.00
25.00
ROOT DISTANCE IN CM

FIGURE 26.

from 2 to 78 hours.

Furthermore, it can be noticed that the existence, at 6 cm from the root on both samples, of an increase followed by a decrease of zinc concentration. This is seen more clearly after a long soaking time, and corresponds to the same feature observed at 10 cm from the root for hairs plucked 4 months later (see Fig. 25). This difference in the position of the region of increased zinc uptake is consistent once more (as in the case of copper) with the length of hair which would have grown during the 4 month period separating the hair acquisition of the two pairs of hairs, given the average growth rate quoted in the literature (0.35 mm/day) (Fle. 54).

Again, the positions of the regions of maximum metal uptake are different for zinc and copper, as is clearly seen in Fig. 27, where the concentration patterns of these two elements are compared for hairs taken simultaneously from subject A.

An experiment where three hairs from subject I were soaked for 136 hours in the same solution was conducted next, to see if such a long period of soaking will attenuate the piece-to-piece and the overall variation in the amount of zinc absorbed. The results shown in Fig. 28 prove that no such attenuation occurs, but instead that the distal end of these three hairs absorb 2 to 3 times more zinc than the region close to the root.

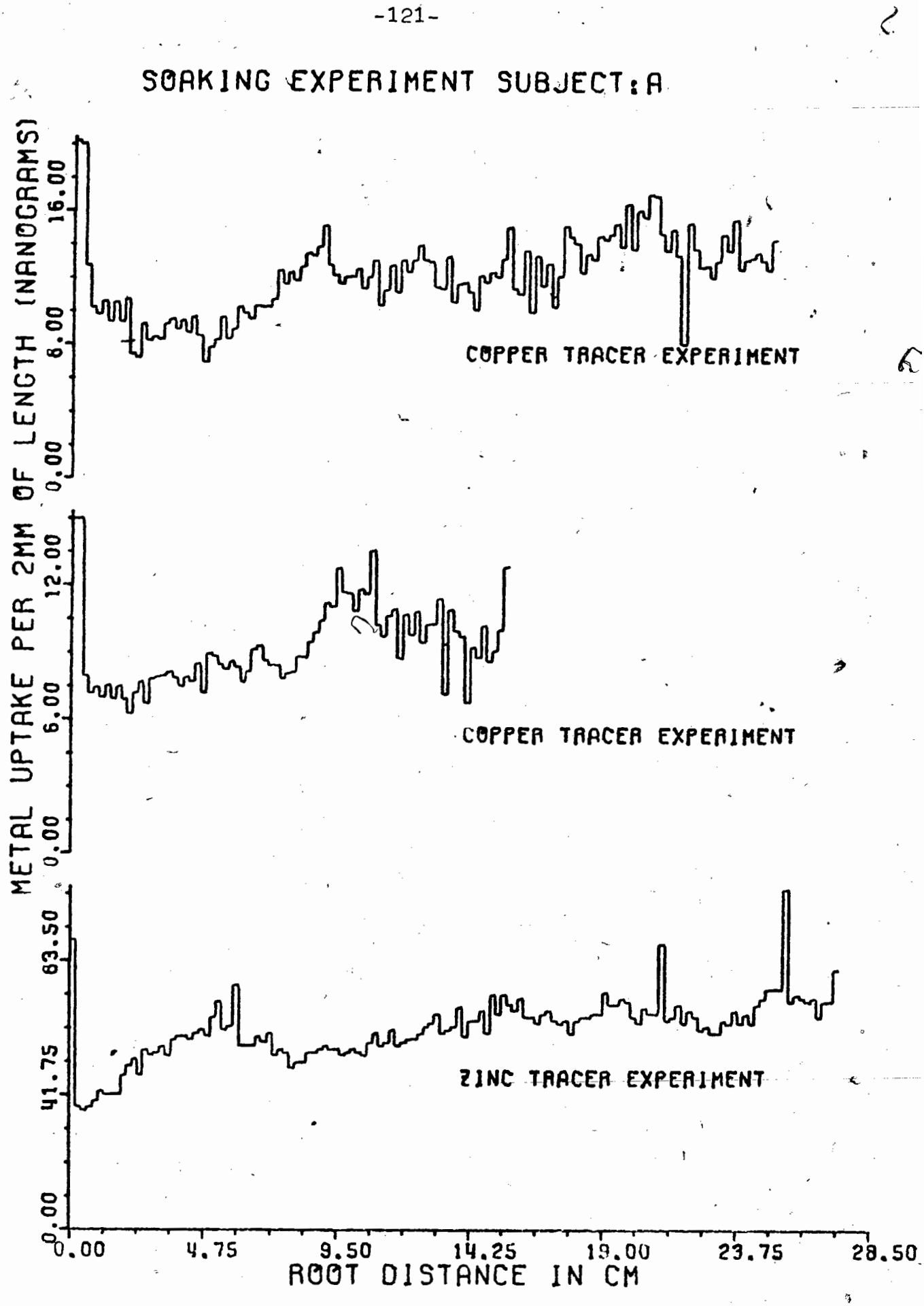

SOAKING EXPERIMENT SUBJECT:A
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
0.00
8.00
16.00
COPPER TRACER EXPERIMENT
0.00
6.00
12.00
COPPER TRACER EXPERIMENT
0.00
41.75
83.50
ZINC TRACER EXPERIMENT
0.00
4.75
9.50
14.25
19.00
23.75
28.50
ROOT DISTANCE IN CM

FIGURE 27

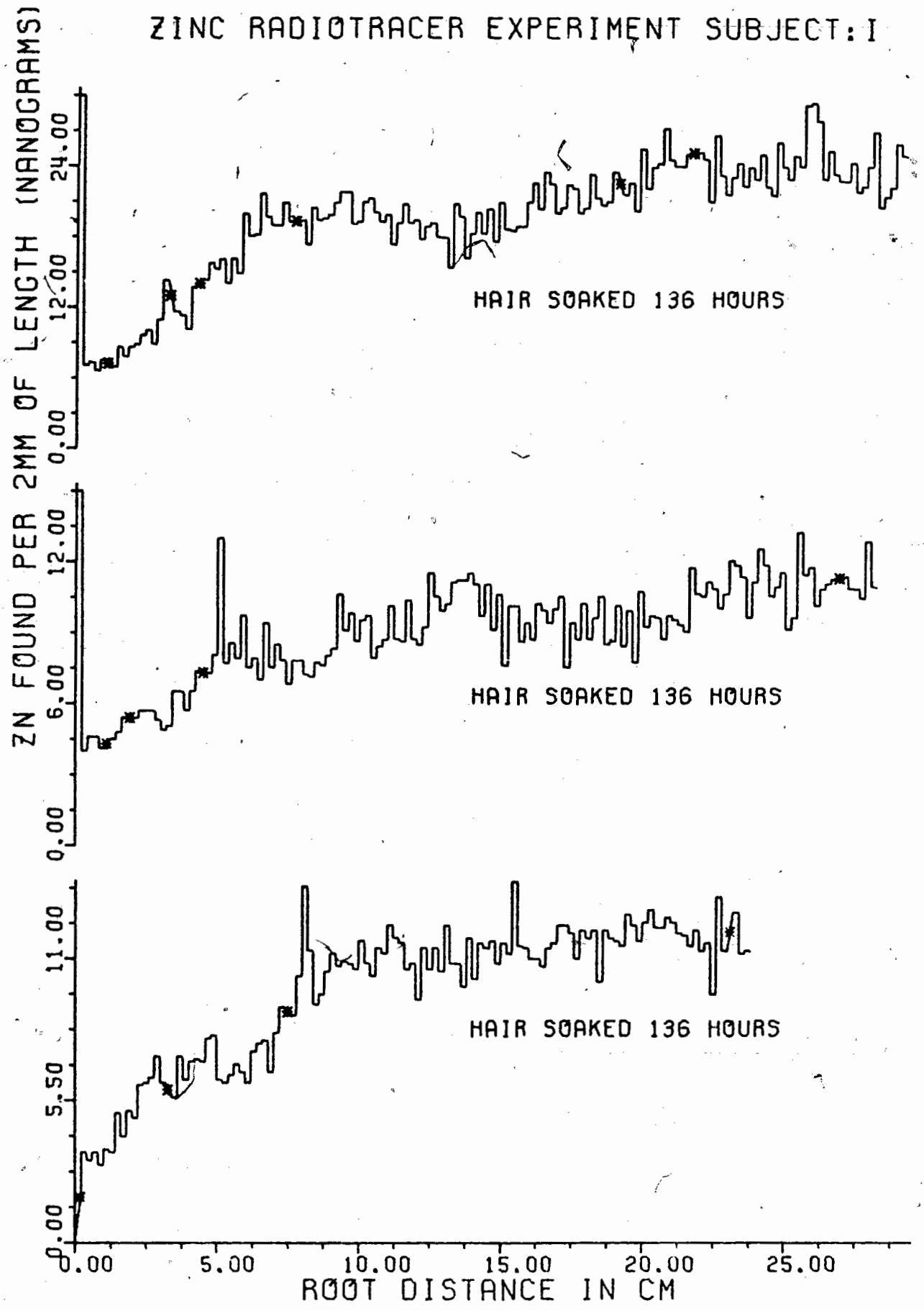

ZINC RADIOTRACER EXPERIMENT SUBJECT: I
ZN FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
12.00
24.00
HAIR SOAKED 136 HOURS
0.00
6.00
12.00
HAIR SOAKED 136 HOURS
0.00
5.50
11.00
HAIR SOAKED 136 HOURS
0.00
5.00
10.00
15.00
20.00
25.00
ROOT DISTANCE IN CM

FIGURE 28

This could be interpreted as a consequence of the variation in the number of "binding sites" available for zinc along the hair, or as being due to an increase of the rate of diffusion of zinc into the keratin in some specific regions of the hair fiber.

As for subject B, no similarities can be found between the concentration patterns of natural and added zinc, which seems to be a significant feature of the behaviour of this metal in hairs.

The characteristic copper concentration patterns obtained with the hairs of subject G, who had her hair partially bleached, suggested an experiment to test if such a feature would be observed with zinc.

One hair from this subject was therefore soaked in the radioactive zinc solution for 1 hour, and it can be seen in Fig. 29 that this metal is absorbed preferentially in the region where the hair was bleached, which is a result similar to the patterns obtained for both natural and added copper. Such a behaviour could be explained again by the same two mechanisms we previously described, and it is not possible at this point to decide if the increased absorption observed in the bleached region could be due to a more open structure of the keratin in this region, which may favour a faster diffusion, or to the presence of an increased number of binding sites for zinc.

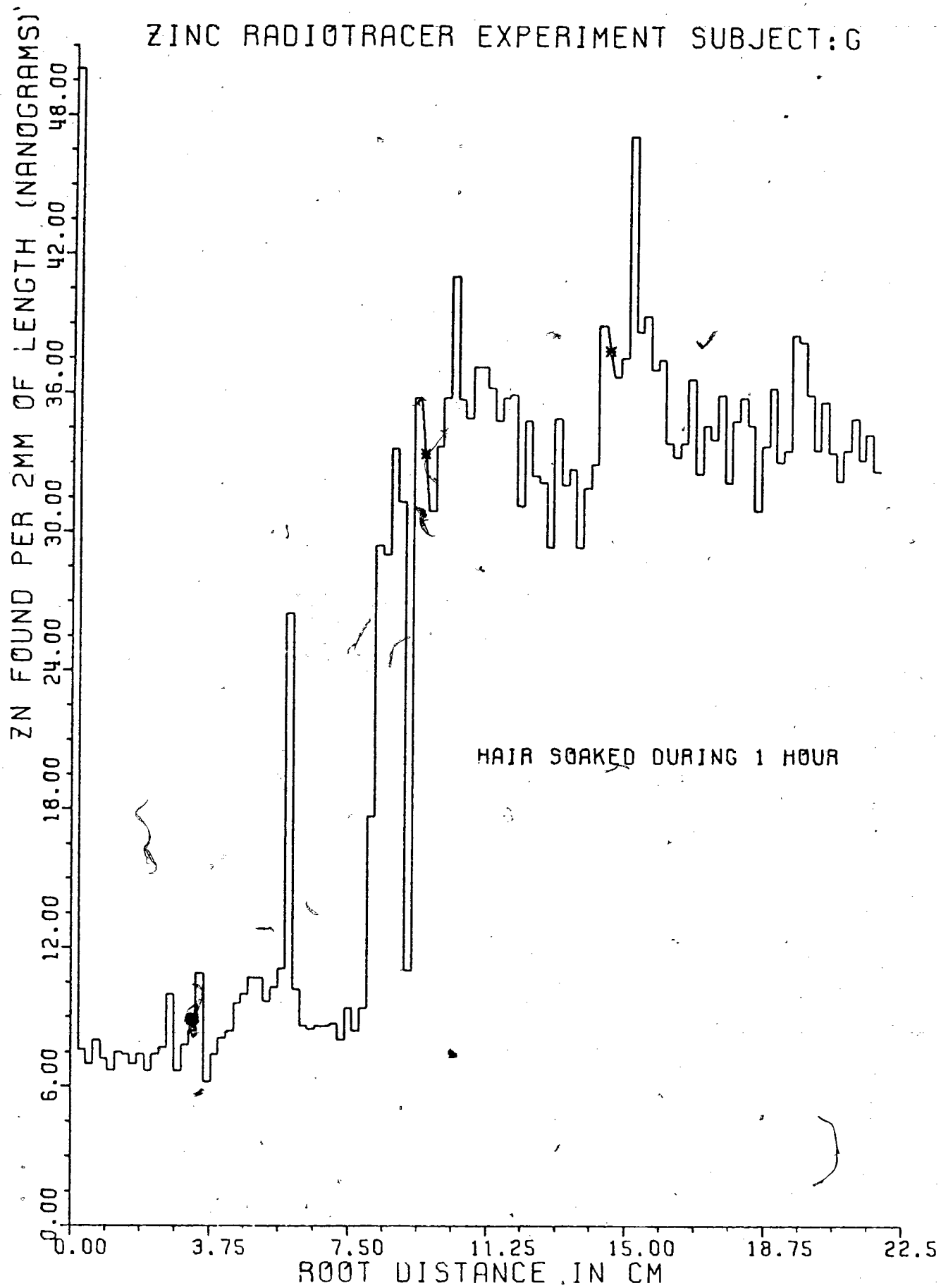

ZINC RADIOTRACER EXPERIMENT SUBJECT:G
ZN FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
6.00
12.00
18.00
24.00
30.00
36.00
42.00
48.00
HAIR SOAKED DURING 1 HOUR
0.00
3.75
7.50
11.25
15.00
18.75
22.50
ROOT DISTANCE IN CM

FIGURE 29

A similar experiment was finally conducted with one hair of subject J, which showed evidence of two zones of discoloration due to bleaching between 5 and 10 cm from the root, and again from 12 cm to the distal end of the hair. The result obtained in Fig. 30 is very similar to that described previously for subject G.

It seemed interesting, for the purpose of comparsion, to see if such hairs will have the characteristic flat concentration pattern for natural zinc obtained with subjects B and I. It is seen in the top of Fig. 31 that the indigenous zinc content of such hair decreases markedly in the bleached region in contrast to the enhanced absorption observed from radiotracer Zn solution. This observation seems to argue against the existence of more binding sites in such regions, but argues for the existence of a more open structure in the bleached area, which could increase the rate of diffusion of the metallic ions into and out from the keratin structure.

It is now possible to interpret the difference observed between the concentration patterns of natural and added zinc for the hairs of subjects B and I.

The results obtained with subject J seem to demonstrate that the natural zinc can be washed out from a region where this metal has been shown to be absorbed preferentially during the soaking experiment.

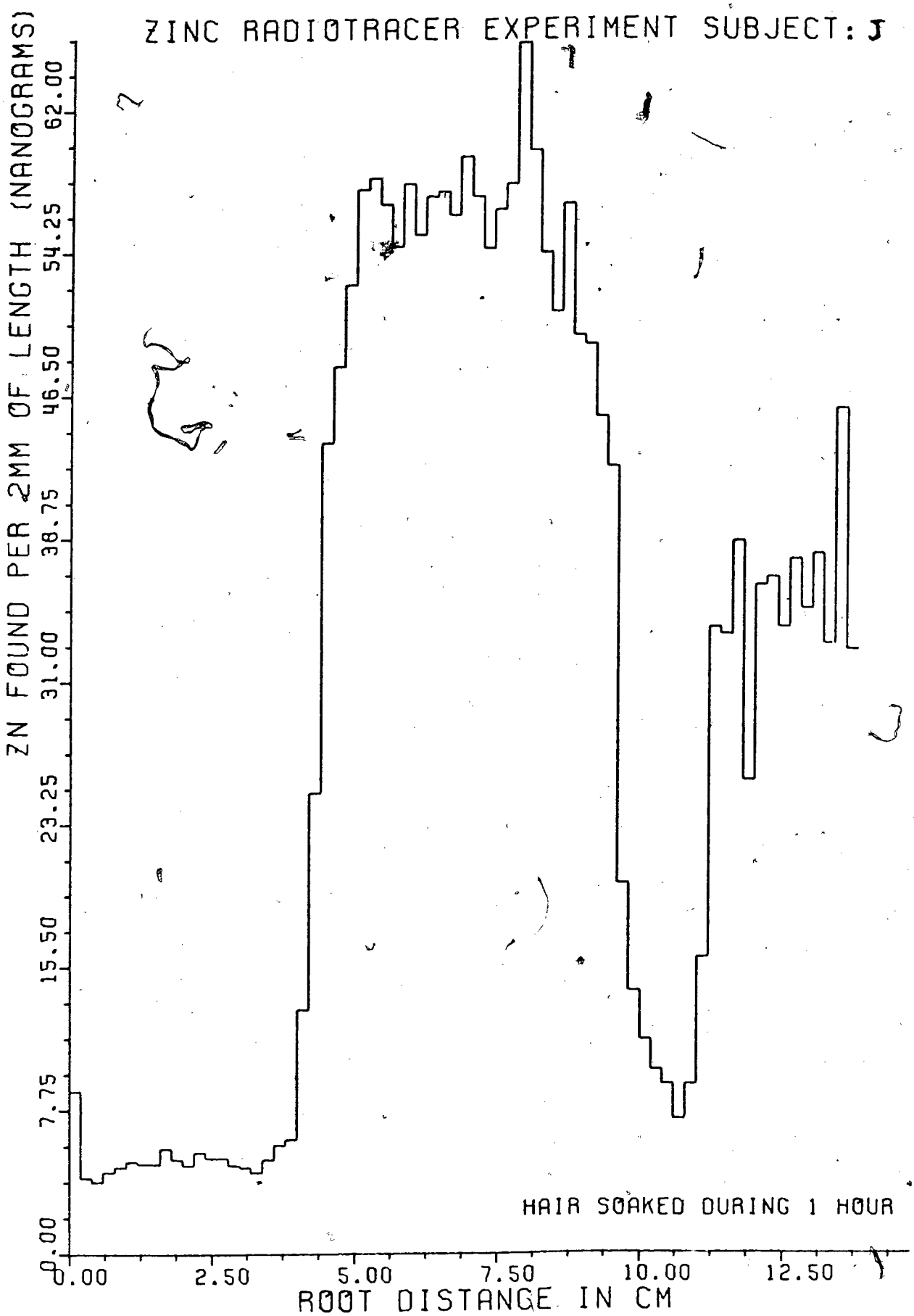

ZINC RADIOTRACER EXPERIMENT SUBJECT: J
ZN FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
7.75
15.50
23.25
31.00
38.75
46.50
54.25
62.00
HAIR SOAKED DURING 1 HOUR
0.00
2.50
5.00
7.50
10.00
12.50
ROOT DISTANCE IN CM

FIGURE 30

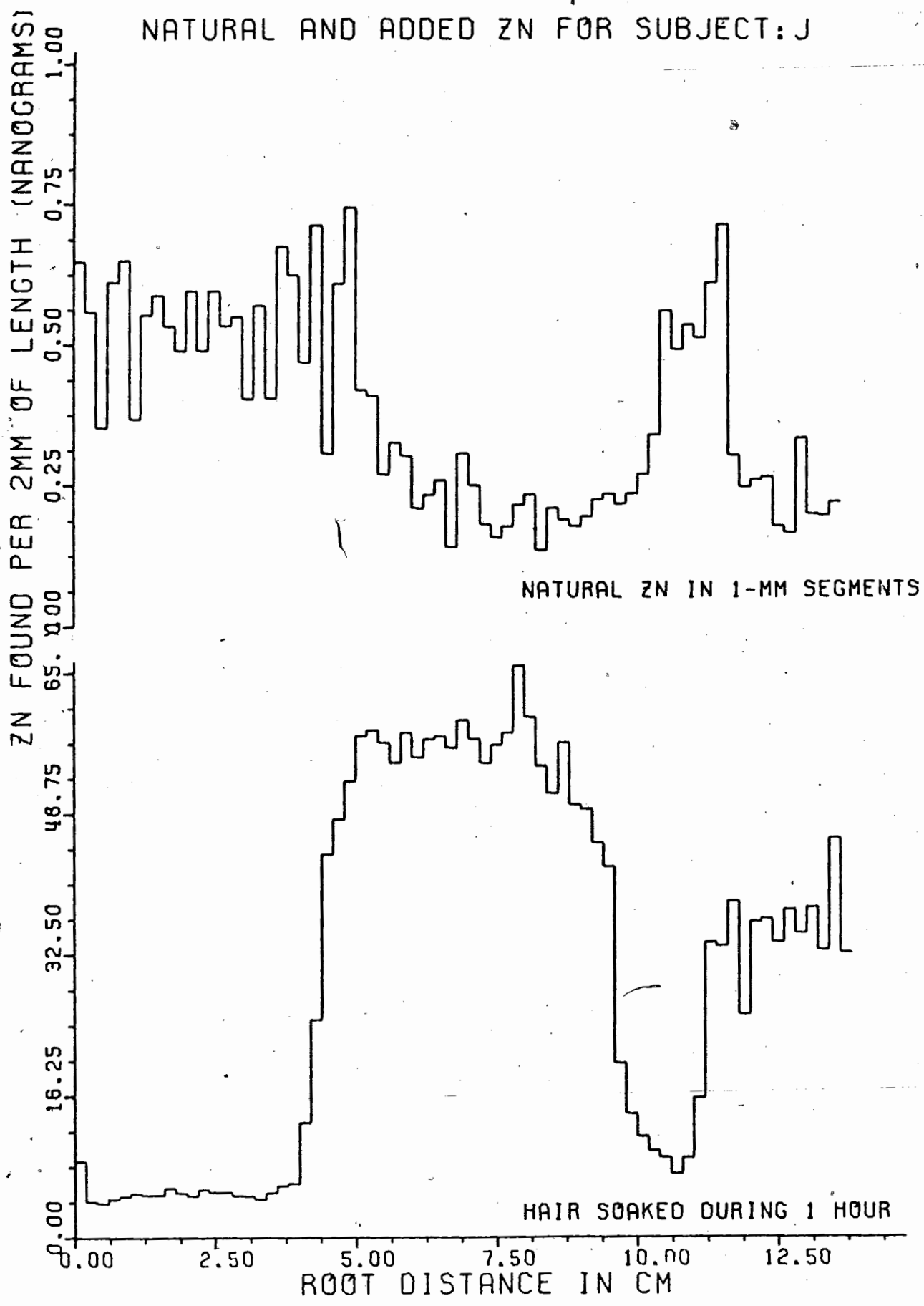

NATURAL AND ADDED ZN FOR SUBJECT: J
ZN FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
0.25
0.50
0.75
1.00
NATURAL ZN IN 1-MM SEGMENTS
0.00
16.25
32.50
48.75
65.00
HAIR SOAKED DURING 1 HOUR
0.00
2.50
5.00
7.50
10.00
12.50
ROOT DISTANCE IN CM

FIGURE 31

Although the region of the hair where this phenomenon has been observed was especially "damaged" by the bleaching treatment, it is reasonable to assume that such a washing out of the natural zinc would also occur generally in the regions of the hair where this metal is preferentially absorbed as, for example, in the case of subjects B and I during the soaking experiment.

The increased removal of the natural zinc from such zones may be due to a more open structure of the keratin (as compared to regions close to the root), which is the result of the ageing and drying of this proteinaceous material. However, incorporation of the indigenous Zn via the follicle is not ruled out.

3.3) Experiments on Cobalt

The characteristics previously described of the behaviour of absorbed zinc and copper in hairs prompted similar experiments with a further transition metal, namely cobalt. The long half-life of ^{60}Co (5.27 years) made this a convenient radiotracer.

3.3.1) Tracer Experiment

One hair fom subject G and one from subject J, both of whom had used a bleaching agent which resulted in very characteristic increases of absorption for both zinc and copper in the discolored region of the

hair, were soaked in a 0.281 mg/ml cobalt solution for 3.5 and 2.5 hours respectively, and then successively rinsed in water, acetone, and ether for 15 seconds each step.

The resulting radioactivity patterns shown in Fig. 32 and 34 seem to sustain the conclusion reached during the zinc and copper tracer experiment. Again, in the bleached regions of the hairs, 2 to 3 times as much cobalt on the average has been absorbed as in the untreated area. However, the pattern obtained now is characterized by large segment-to-segment variation in the quantity of metal absorbed (especially in the bleached region), resulting in a less clear-cut difference in behaviour of the bleached section from that of the remaining part of the hair.

It can also be noticed that while a soaking of 1 hour in a 0.1 mg/ml solution of zinc resulted in the bleached region absorbing 6 times more Zn than the unbleached hair, a 3.5 hour soaking in a 0.28 mg/ml cobalt solution produced a factor of only ? in the relative Co concentrations.

Furthermore, the average amount of cobalt absorbed by each 2-mm hair segment is less than the corresponding quantity for zinc and copper. This conclusion is in agreement with the finding of Bate (Bat. 65), who found 10 times more zinc than cobalt was absorbed in hairs at a pH of 5.5.

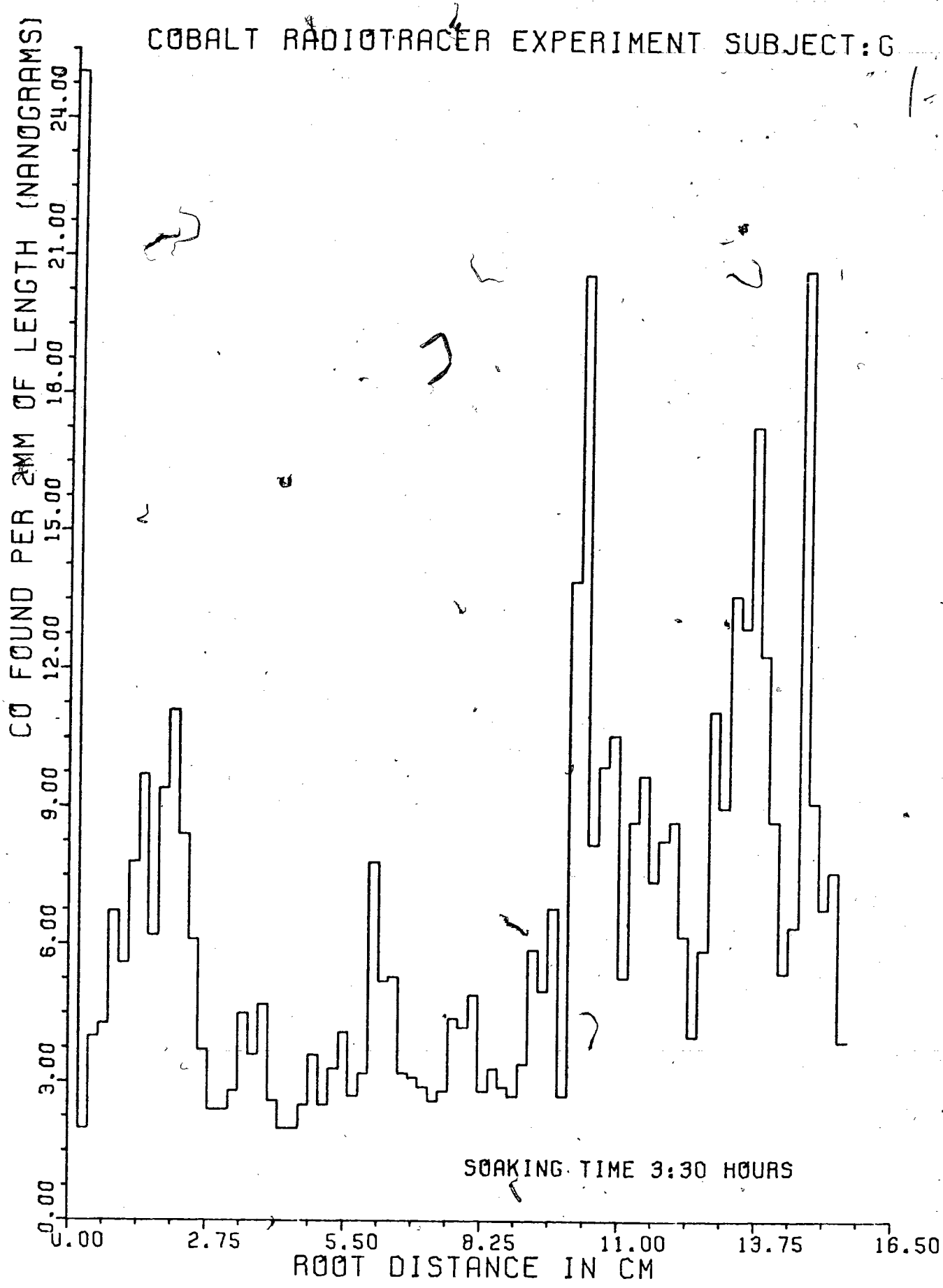

COBALT RADIOTRACER EXPERIMENT SUBJECT:G
CO FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
3.00
6.00
9.00
12.00
15.00
18.00
21.00
24.00
SOAKING TIME 3:30 HOURS
0.00
2.75
5.50
8.25
11.00
13.75
16.50
ROOT DISTANCE IN CM

FIGURE 32

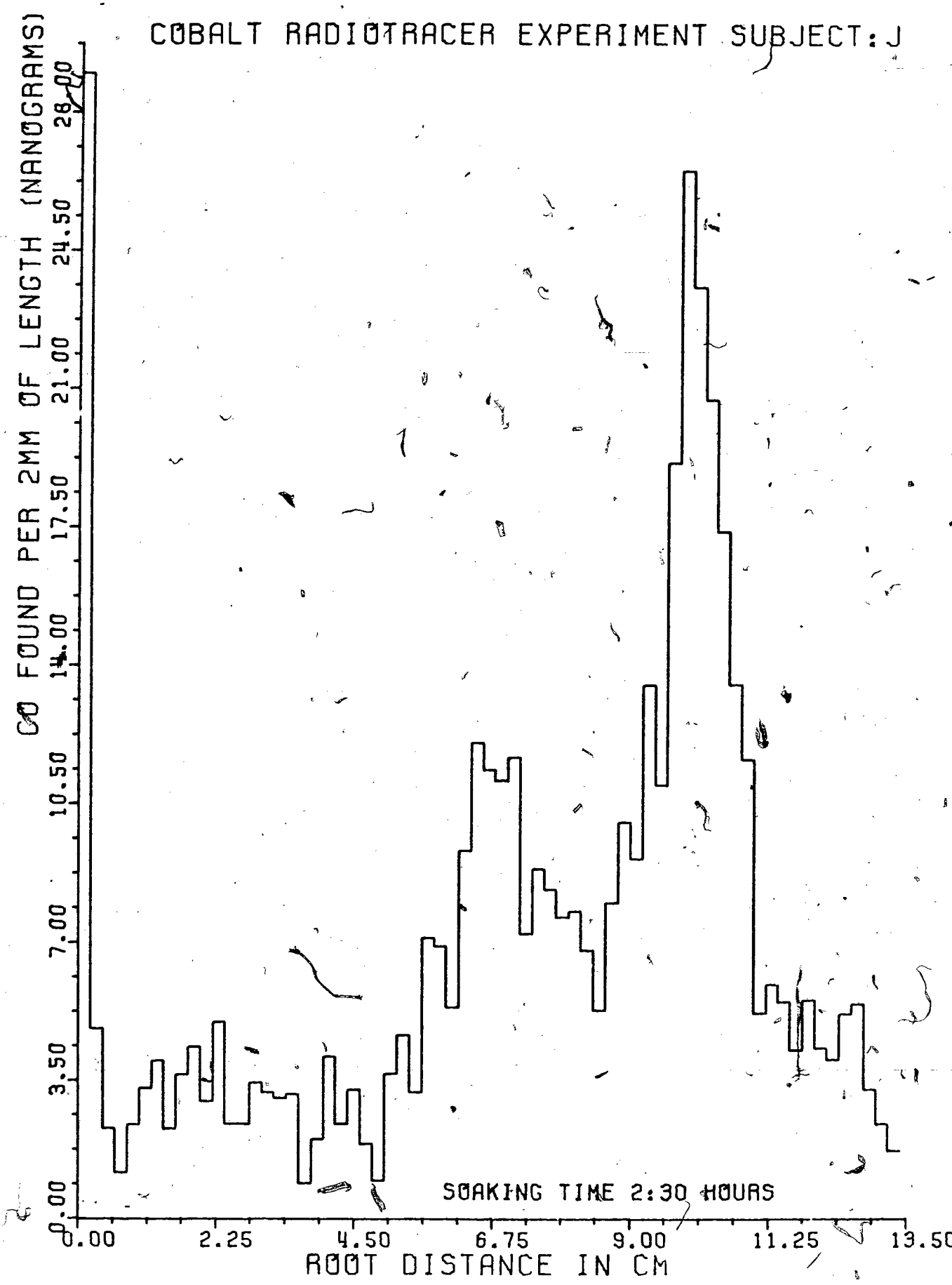

COBALT RADIOTRACER EXPERIMENT SUBJECT: J
CO FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
3.50
7.00
10.50
14.00
17.50
21.00
24.50
28.00
SOAKING TIME 2:30 HOURS
0.00
2.25
4.50
6.75
9.00
11.25
13.50
ROOT DISTANCE IN CM

FIGURE 34

The influence of increased soaking time on the amount of Co absorbed, and the effect of subsequent rinsing on the concentration pattern, were investigated in an experiment, the results of which are shown in Fig. 33.

Three hairs taken from subject G were soaked for 91 hours in the above cobalt solution, and rinsed successively in water, acetone, and ether for the different periods of time given in the figure. It can be seen that no significant increase in the level of cobalt absorbed can be noticed, even when the soaking time is increased to 91 hours.

In this, the behaviour of cobalt seems to be different from that observed for zinc and copper, for which absorption was seen to increase for prolonged soaking times. This could be attributed to a possible limitation in the diffusion of cobalt to the outside region of the hair (i.e. the cuticle), with an accompanying saturation of the keratin in this region after a short soaking time, because of the precipitation of colloidal cobalt hydroxide whose molecular dimension prevented penetration further in the hair structure. The influence of the time of rinsing is seen to be similar to that observed for copper, a diminution of the quantity of Co absorbed and also the segment-to-segment fluctuations, together with a smaller difference between the cobalt content of the bleached region of the hair and elsewhere. It can be noticed that, for the three hairs whose patterns

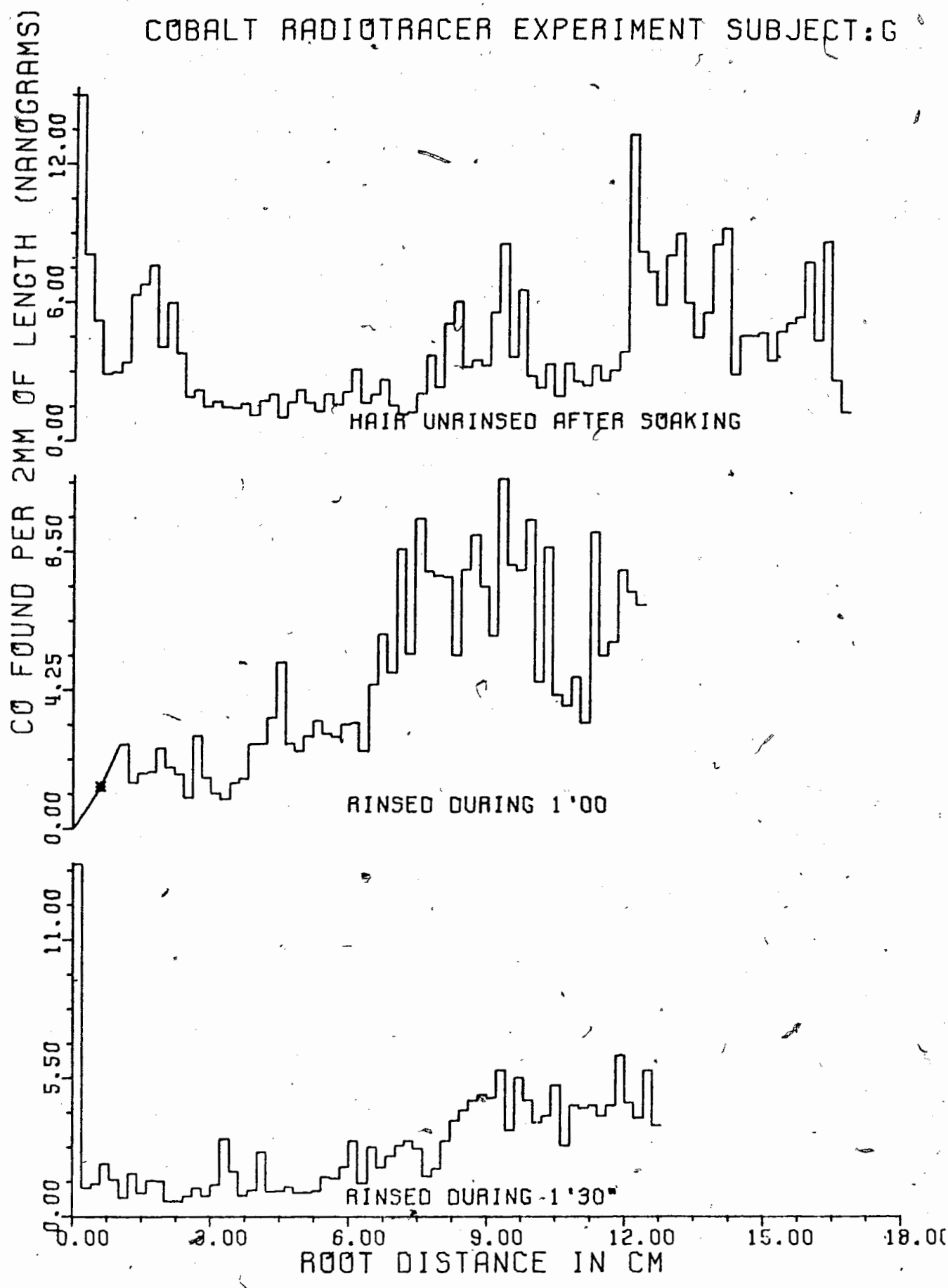

COBALT RADIOTRACER EXPERIMENT SUBJECT:G
CO FOUND PER 2MM OF LENGTH (NANOGRAMS)
0.00
6.00
12.00
HAIR UNRINSED AFTER SOAKING
0.00
4.25
8.50
RINSED DURING 1'00
0.00
5.50
11.00
RINSED DURING 1'30"
0.00
3.00
6.00
9.00
12.00
15.00
18.00
ROOT DISTANCE IN CM

FIGURE 33

are shown in Fig. 33, the position of the region of increased concentration seems to vary markedly from sample to sample. This was evidently due to the bleached regions occurring in different positions on different hairs, a circumstance verified via other hair specimens taken from the same subject.

3.4) Experiment on Arsenic

The analysis of the arsenic content of human hairs has always attracted forensic scientists because of its applicability to the detection of poisoning.

Strongly diverging theories exist as to whether the arsenic seen in the hair originated from incorporation via the follicle during the growth process (in which case it would be possible to ascertain a date of acute poisoning (Pea. 71)), or is deposited along the hair shaft by the sweat and the spreading action of washing habits (Lim. 66). In the latter case, it would be evidently impossible to interpret any variation of arsenic concentration along the length of the hair as being due to a possible chronic poisoning, since after a long period of time, the arsenic would have migrated over the whole length of the hair.

The interesting results described by Lima (Lim. 66) on the movement by capillary action of arsenic along the surface of the hair

demonstrate such a migration. In the light of the concentration patterns observed in the present work for other elements, it seemed necessary to verify if the presence of regions of high arsenic concentration (which were reported along hairs analysed by Neutron Activation Analysis (Smi. 64)) may have originated from the absorption of As from external sources onto zones of high absorption capacity along the hair length.

3.4.1) Tracer Experiment

As for zinc, copper, and cobalt, the detection of regions of high absorption capacity was effected by soaking different hair samples for variable amounts of time, in a 0.58 mg/ml and 0.2 mci/ml radioactive arsenic solution with the pH adjusted to 5.5.

One hair from subject A was soaked for 22 hours and the arsenic radioactivity was assayed by the usual method. The results are shown in Fig. 35, where the pattern described is markedly different from that obtained previously when metals such as copper and zinc were absorbed on hairs taken at the same time from the same subject.

In particular, the amount of arsenic absorbed on each 2-mm segment is seen to decrease with the distance from the root, whereas the natural copper content of this subject's hairs increases sharply at 20 cm from the root (Fig. 2).

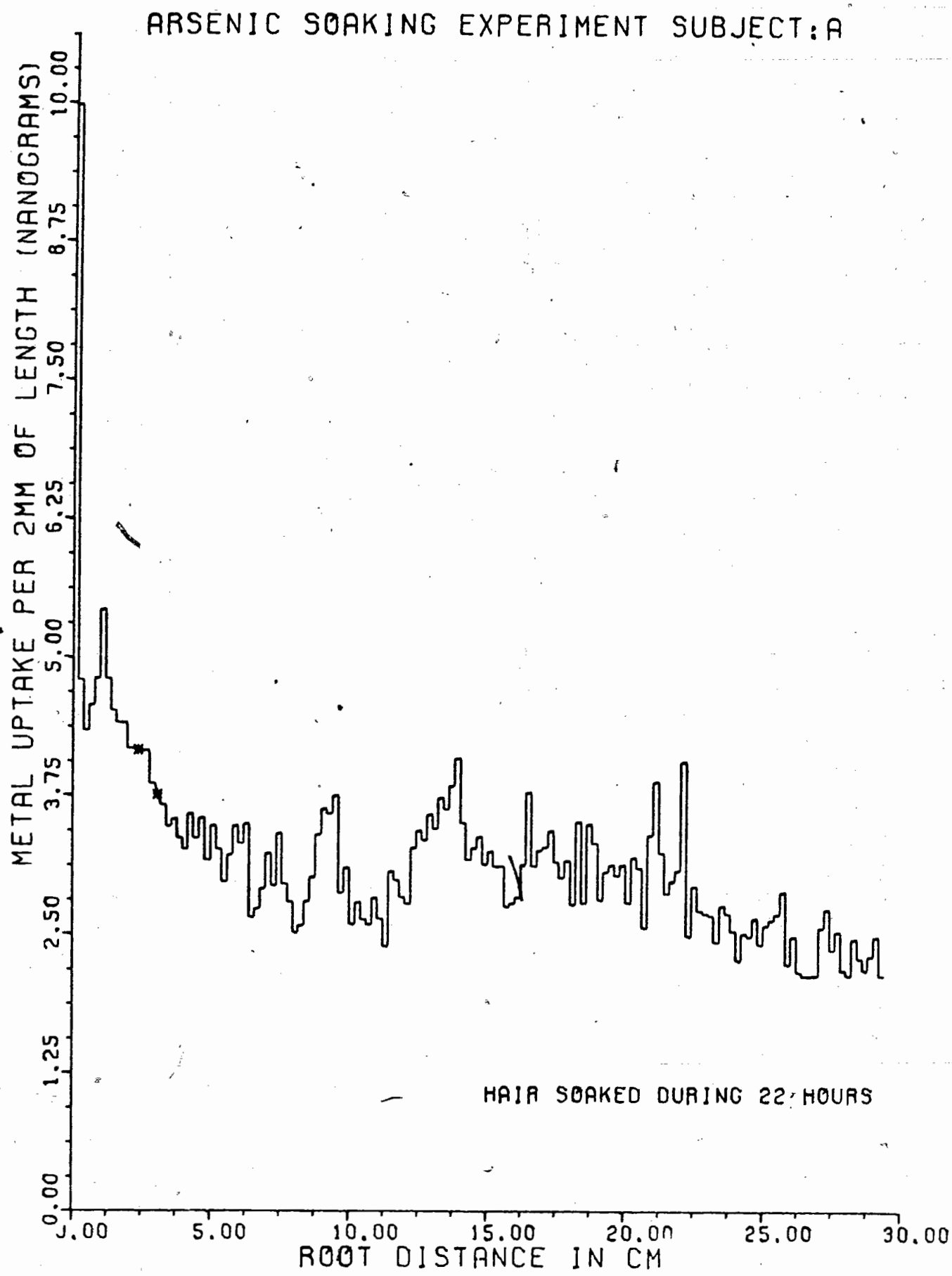

ARSENIC SOAKING EXPERIMENT SUBJECT:A
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
0.00
1.25
2.50
3.75
5.00
6.25
7.50
8.75
10.00
HAIR SOAKED DURING 22 HOURS
0.00
5.00
10.00
15.00
20.00
25.00
30.00
ROOT DISTANCE IN CM

FIGURE 35

Furthermore, from Fig. 36 where the added concentration patterns of zinc and arsenic of such hairs are shown, it is seen that one region of maximum zinc absorption, which was situated around 10 cm from the root, corresponds to a zone of somewhat reduced arsenic absorption.

This phenomenon may be related to the results of Bate (Bat. 66a) who showed that elements such as As, Se, and P were absorbed in hairs to a lesser extent when the pH of the soaking solution was raised from 3.5 to 5.5, in contrast to Cu, Zn, and Co, which were absorbed more strongly. Therefore, it seems that a correlation may exist between the absorption of elements such as Cu, Zn, and Co (and As) in hairs and their chemical properties, which is a point we will dicuss later.

In view of such a particular behaviour, it was decided to extend this experiment with arsenic to hairs of subject B, which were soaked in the arsenic solution for 14 hours.

It can be seen in Fig. 37 that very unusual features characterize the patterns now observed. In particular, each sample possesses three distinct regions where the arsenic is strongly absorbed from the solution, whose position along the hair length seems to coincide relatively well from hair to hair. Patterns such as this observed for indigenous arsenic in hair might be erroneously interpreted as indicating a date when poisoning of the subject had occurred.

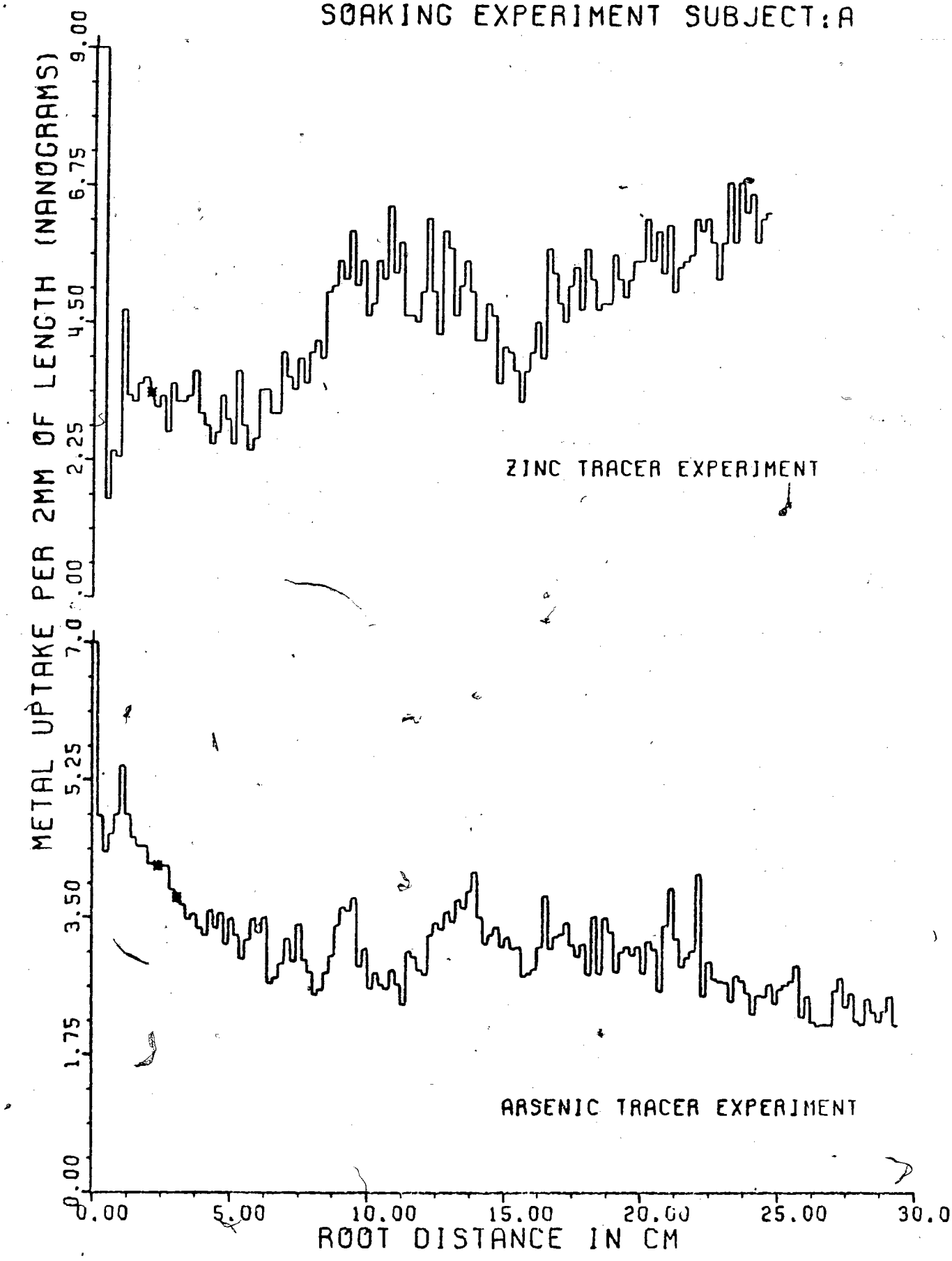

SOAKING EXPERIMENT SUBJECT:A
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
9.00
6.75
4.50
2.25
.00
ZINC TRACER EXPERIMENT
7.0
5.25
3.50
1.75
0.00
ARSENIC TRACER EXPERIMENT
0.00
5.00
10.00
15.00
20.00
25.00
30.00
ROOT DISTANCE IN CM

FIGURE 36

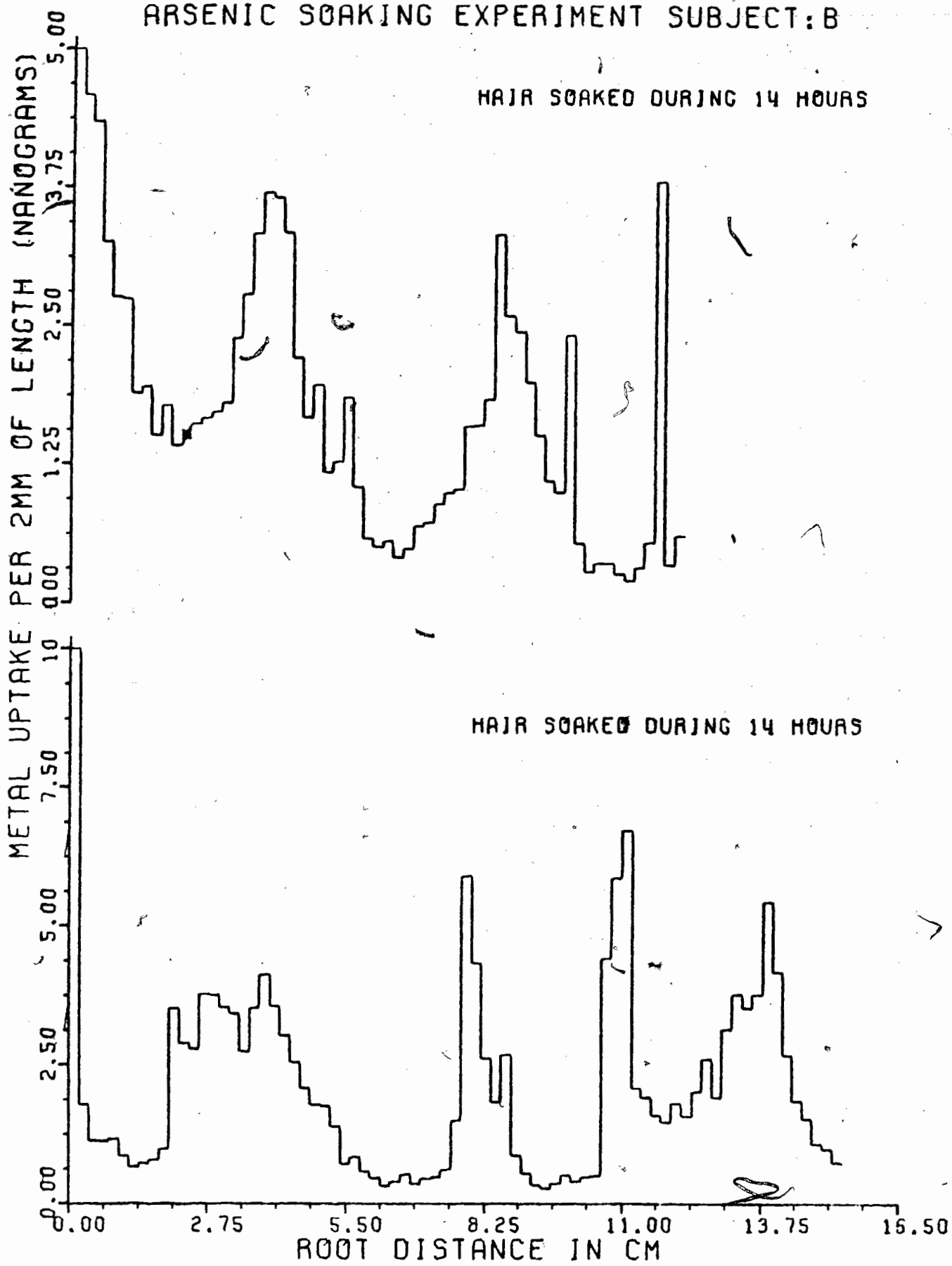

ARSENIC SOAKING EXPERIMENT SUBJECT:B
HAIR SOAKED DURING 14 HOURS
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
5.00
3.75
2.50
1.25
0.00
HAIR SOAKED DURING 14 HOURS
10
7.50
5.00
2.50
0.00
0.00
2.75
5.50
8.25
11.00
13.75
16.50
ROOT DISTANCE IN CM

FIGURE 37

These patterns do not show correlation with the ones shown in Fig. 22 for zinc on the same subject, confirming the above result for hairs of subject A.

A similar experiment was conducted with one hair drawn from subject I. Figure 38 shows the result of the variation of the amount of arsenic absorbed per 2-mm length after a soaking of 14 hours, namely a pattern similar to those for subject B. Again no correlation can be found between this result and the concentration patterns of added zinc on the hairs from the same subject we already described in Fig. 38.

The observation for the hairs of subject A of a decrease in the amount of arsenic absorbed in each 2-mm segment, when for both zinc and copper the absorption was shown to increase along the length of the sample, suggested a similar test on hairs from a subject who exhibited a constant absorption pattern for these two metals. The hairs from subject E were chosen because of this particularity, as we have already seen in Fig. 14. Two of these hairs were soaked for 14 hours in the above arsenic solution, and the patterns obtained are shown in Fig. 39. It can be seen that they are similar, and other than a slight decrease in the amount of arsenic absorbed in the vicinity of the root, the two patterns shown can be considered as practically flat. The hairs of this subject are therefore characterized by the absence of zones of increased or decreased

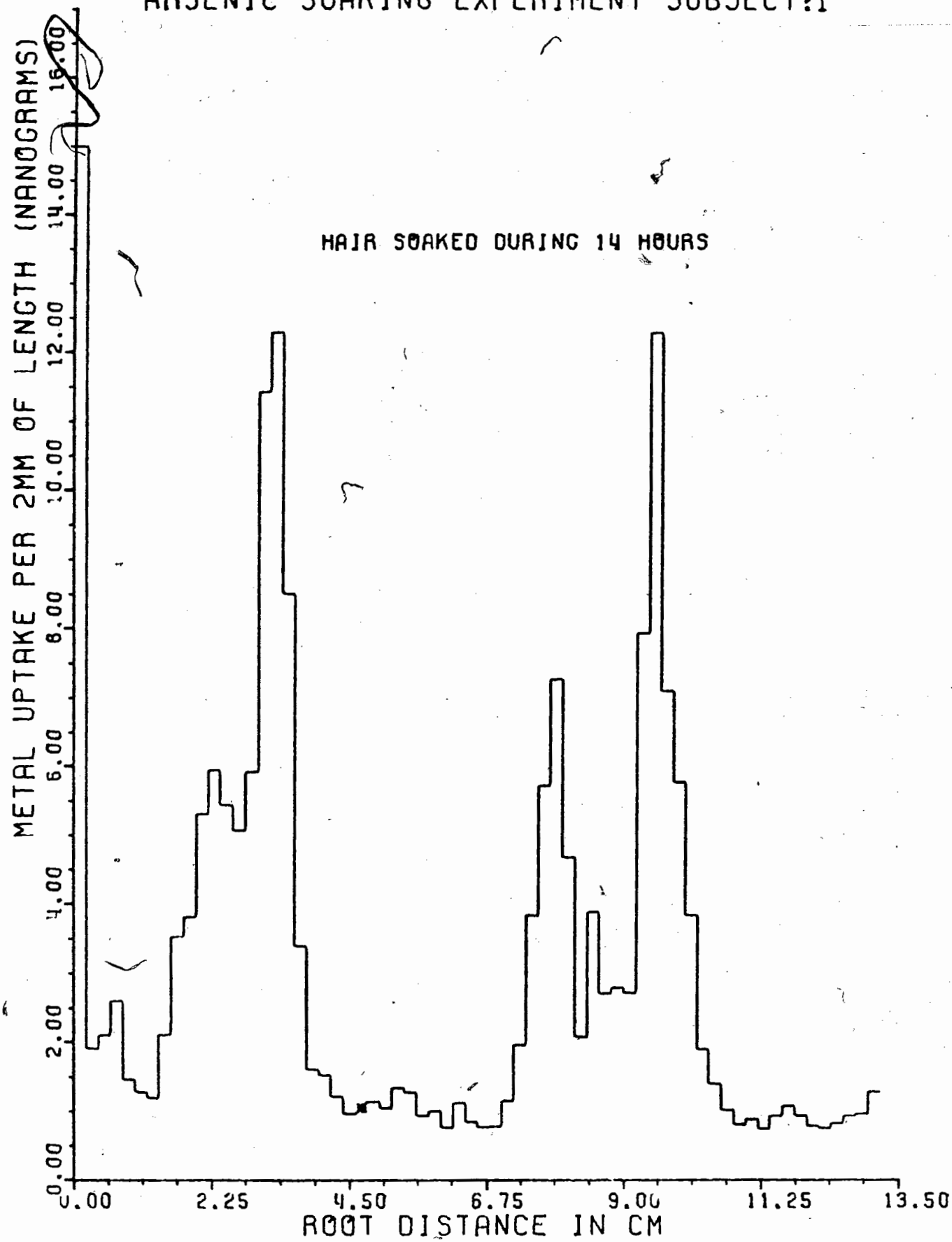

ARSENIC SOAKING EXPERIMENT SUBJECT:I
HAIR SOAKED DURING 14 HOURS
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
0.00
2.25
4.50
6.75
9.00
11.25
13.50
ROOT DISTANCE IN CM

FIGURE 38

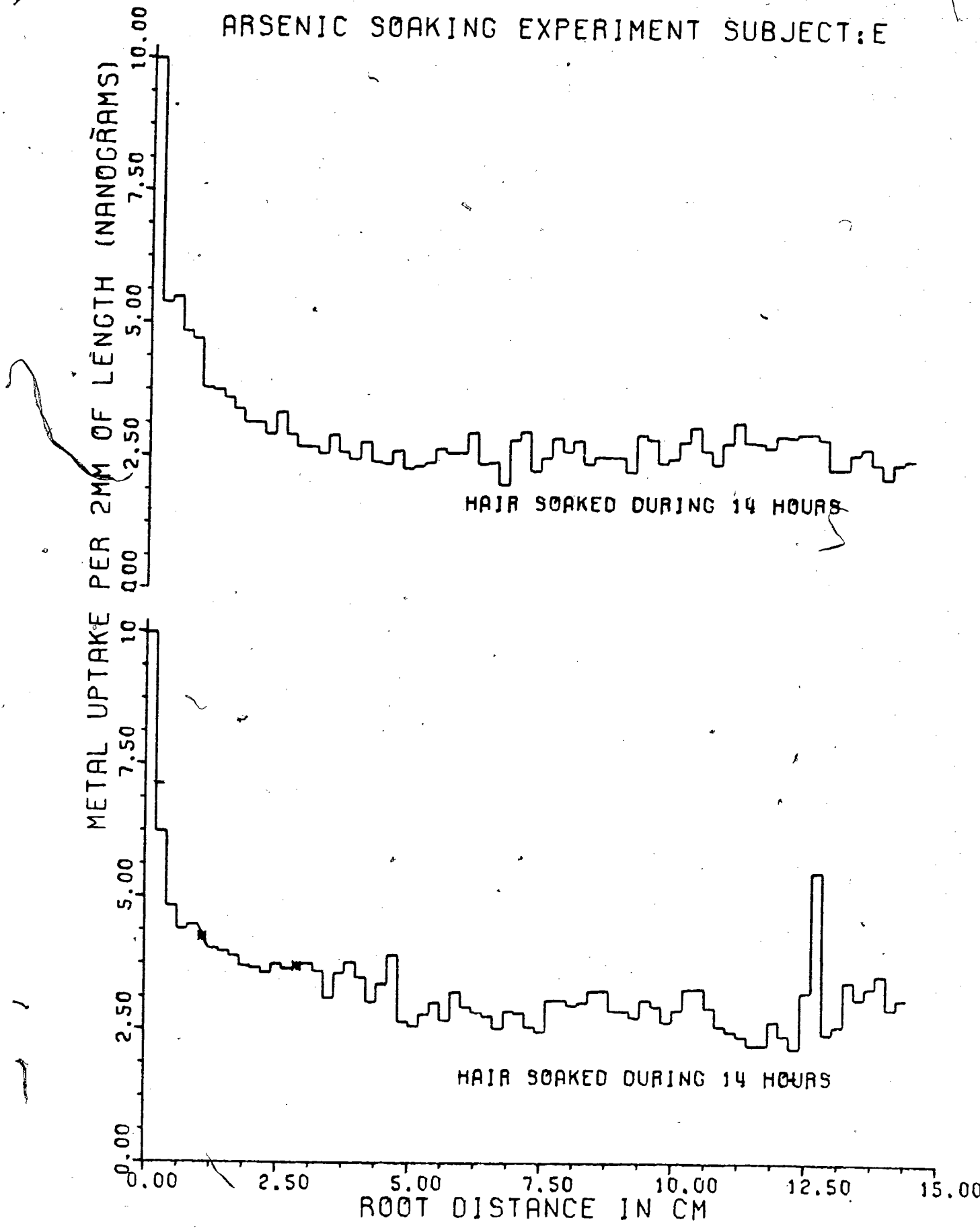

ARSENIC SOAKING EXPERIMENT SUBJECT:E
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
10.00
7.50
5.00
2.50
0.00
HAIR SOAKED DURING 14 HOURS
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
10
7.50
5.00
2.50
0.00
HAIR SOAKED DURING 14 HOURS
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 39

absorption either of metallic ions such as Cu, or of non-metals such as As.

Additional experiments were conducted with one hair of subject H, who had used a bleaching agent on her hair, resulting in a bleached region from 13 cm from the root outwards.

The added arsenic concentration pattern is shown in the bottom of Fig. 40, where a sharp decrease in the amount of arsenic absorbed can be noticed in the bleached region, which was on the other hand observed to absorb copper, zinc, and cobalt more strongly than elsewhere. This behaviour is in agreement with what has been observed so far as the difference between the behaviour of arsenic and the other elements investigated is concerned.

To conclude this series of experiments, one hair from the same subject, but taken after this individual had her hair redyed, was submitted to a soaking in the same arsenic solution. The result shown in the top part of Fig. 40 indicates again a sharp decrease in the amount of arsenic absorbed at approximately the same distance from the root as for the undyed hair. However, the sharp increase of arsenic content observed at 4 cm from the root is unexplained.

In view of the patterns obtained for at least two of the subjects (B and I) whose hairs were submitted to soaking in an arsenic

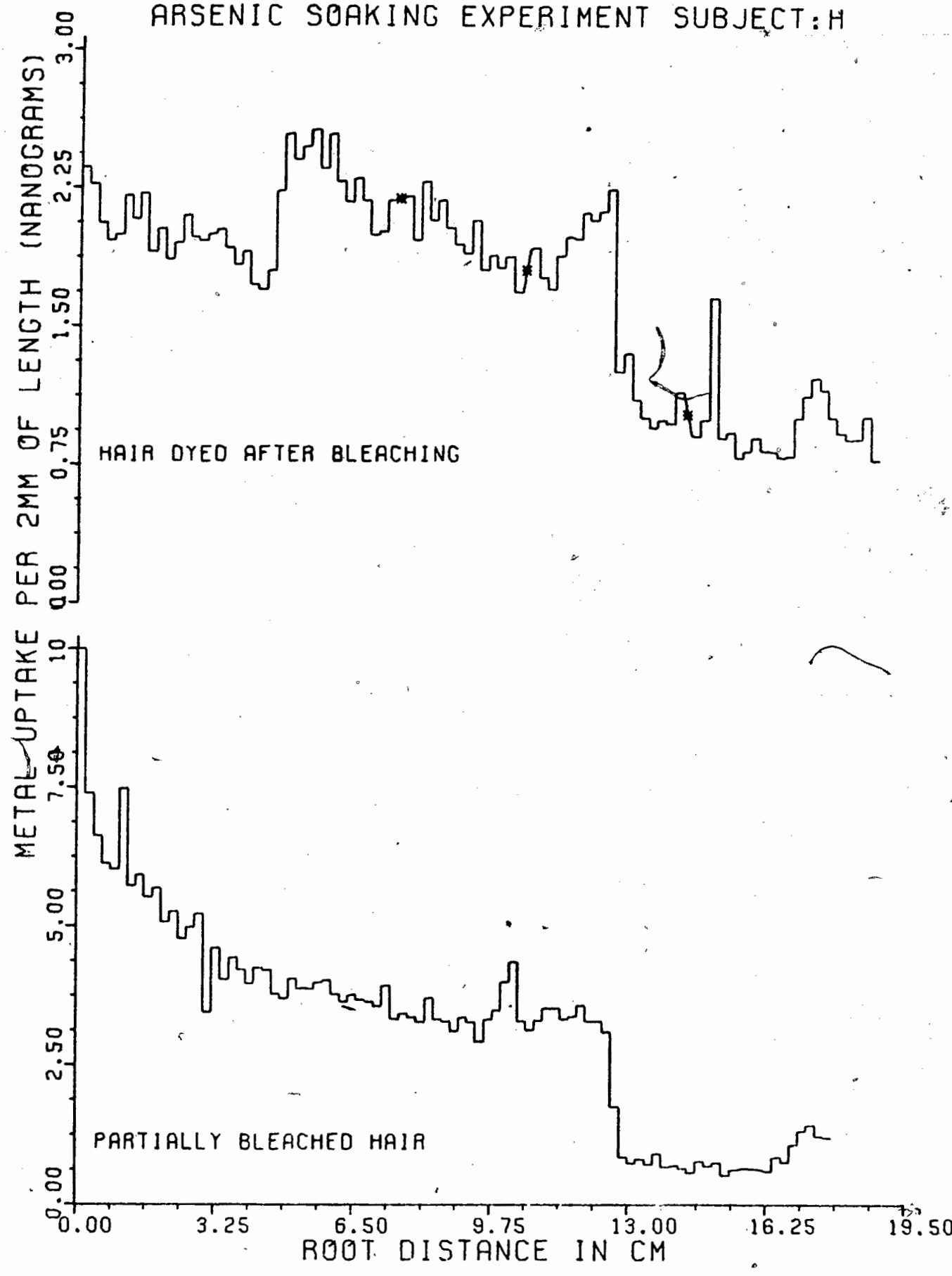

ARSENIC SOAKING EXPERIMENT SUBJECT:H
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
3.00
2.25
1.50
0.75
0.00
HAIR DYED AFTER BLEACHING
10
7.50
5.00
2.50
0.00
PARTIALLY BLEACHED HAIR
0.00
3.25
6.50
9.75
13.00
16.25
19.50
ROOT DISTANCE IN CM

FIGURE 40

solution, it can be concluded that the interpretation of maxima in the concentration pattern of natural arsenic as being caused by possible poisoning on a particular date is subject to suspicion, since this tracer experiment has revealed the existence of zones of preferential arsenic uptake. These would undoubtedly absorb more arsenic from the environment or from sweat (perhaps with an elevated As content following poisoning) creating maxima in the natural concentration of this element, at locations on the hair unconnected with the time of As administration.

Furthermore, the consistent decrease of arsenic uptake where the absorption of zinc was seen to increase may be caused by the possible washing out of arsenic from these regions because of their more open structure, or by a different character of the available binding sites.

3.5) Multi-element Soaking Experiments

The interpretation of tracer absorption patterns in terms of varying concentrations of chemically specific sites perhaps could be subjected to additional test by means of experiments in which hairs were subjected to soaking in more than one tracer at a time, so that competition for sites might occur.

Such experiments, however, posed the problems of separate assay of the tracers used (either via differential decay or γ-spectroscopy),

and of accomplishing such measurements in the face of the half-lives of the available tracer nuclides.

3.5.1) Soaking Experiments With Two Tracers

Because of the interesting and extensive results already obtained from the copper soaking experiments, it was desirable to include this element in a multiple tracer experiment. However, due to the short half-life of the most convenient tracer (12.8-hours, ^{64}Cu), it was not possible to accomplish the necessary extensive program of γ-spectroscopy needed to define a hair absorption pattern with a tracer mixture containing this nucleide, which furthermore does not possess any strong γ-rays.

Thus, a differential decay experiment was the remaining possibility, which dictated the use of a binary mixture of ^{64}Cu and one other nuclide with a very different half-life so that the two activities could be easily separated.

The second component chosen was ^{203}Hg, since this element was known to be strongly sorbed on hair and thus may act as a strong competitor for copper. Indeed, mercury absorption was found to be relatively strong, so that some difficulty was experienced in making meaningful copper measurements against the background represented by the mercury activity. Only in the case of one hair from subject A

(soaked for 23 hours in a solution at pH 4.5) was this really possible, and the results are shown in Fig. 41.

It will be recalled that for this subject, the copper absorption pattern (seen in Fig. 11) is highly structured. The copper pattern in Fig. 41 is much less structured; some increase in copper concentration from the root to the distal end is still observed. Similarly, the amount of mercury absorbed increases very slightly along the hair, but to a far lesser extent than either copper or, for example, zinc on hair samples taken at the same time from the same subject.

It seems, then, that the presence of mercury in the soaking solution may have interfered with the absorption of copper into the keratin, since the pattern characteristic of this element (Fig. 2) in these specific hairs seems to have been markedly modified.

This point was confirmed during the five-element soaking experiment.

3.5.2) Five-element Soaking Experiment

Following this observation, and to conclude this whole series of experiments on trace element concentration patterns in human hairs, competition experiments were conducted with five tracers mutually compatible for the purposes of γ-spectroscopic assay, namely ^{203}Hg,

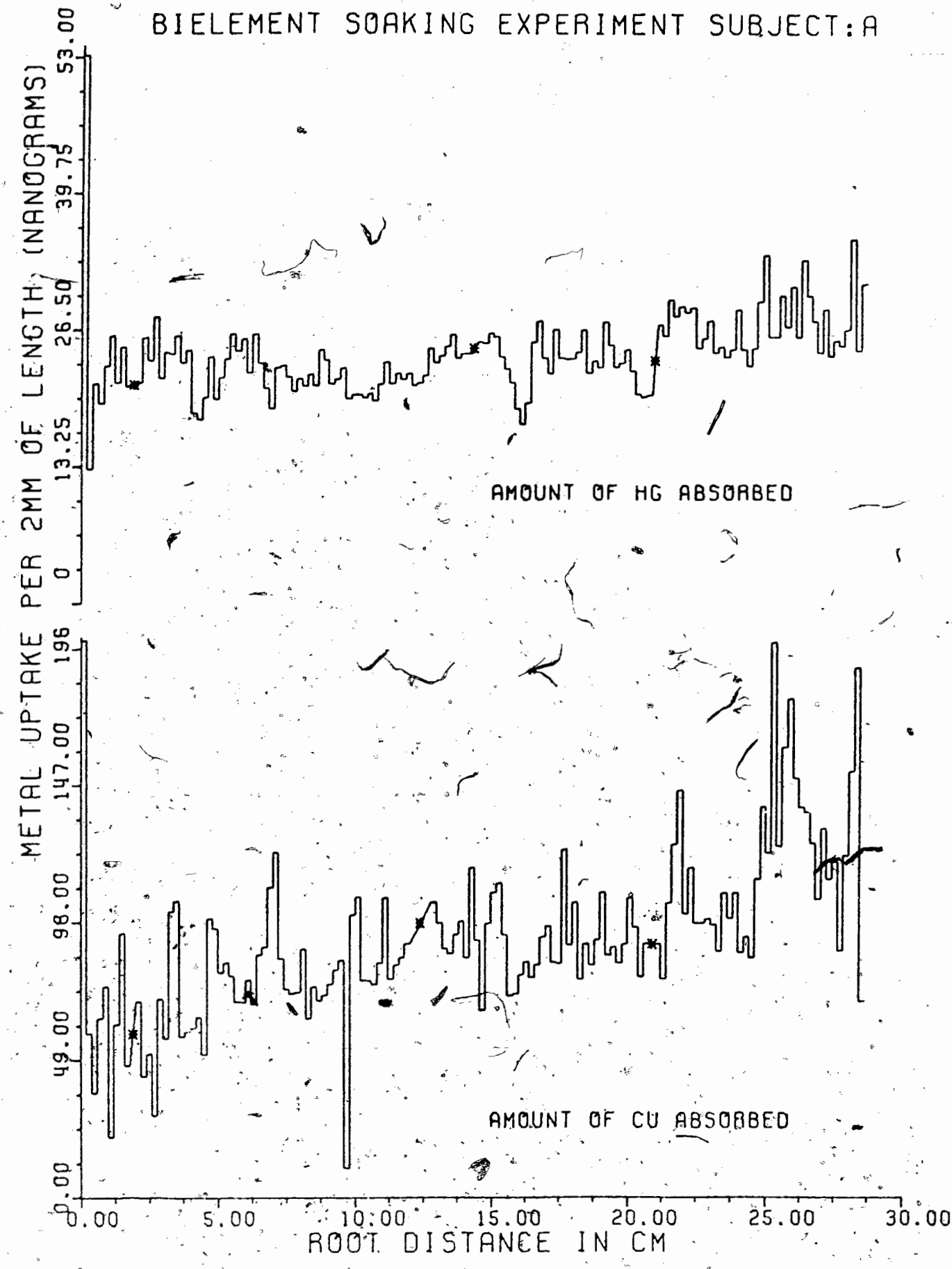

BIELEMENT SOAKING EXPERIMENT SUBJECT:A
METAL UPTAKE PER 2MM OF LENGTH (NANOGRAMS)
0
13.25
26.50
39.75
53.00
AMOUNT OF HG ABSORBED
0.00
49.00
98.00
147.00
196
AMOUNT OF CU ABSORBED
0.00
5.00
10.00
15.00
20.00
25.00
30.00
ROOT DISTANCE IN CM

FIGURE 41

^{124}Sb, ^{65}Zn, ^{110m}Ag, and ^{60}Co. Despite the interesting results obtained with arsenic, it was not possible to include this element because of its short half-life (26.3 hours). It was decided, however, to replace arsenic by antimony, which has to some extent similar chemical properties, and copper by silver for similar reasons.

One hair from subject A was soaked in the mixed solution for 23 hours, and the different patterns obtained for each element are shown in Fig. 42. The patterns for all five elements are seen to be rather flat and featureless, although a slight increase in the amount of antimony absorbed on each 5-mm segment can be observed as a function of distance from the root.

It is interesting to compare the concentration pattern of zinc particularly with the one shown in Fig. 25 for zinc absorbed by itself into a hair taken from the same subject at the same time.

It is clear that, as in the case of the influence of mercury on the copper pattern discussed above, the presence of the other elements now sorbed on the hair at the same time as zinc is responsible for suppression of the highly structured zinc pattern previously observed, evidently by competing for, or saturating, the binding sites responsible. It seemed interesting to verify this conclusion with hairs from another subject (subject F) whose copper concentration patterns were also known to contain characteristic features such as a

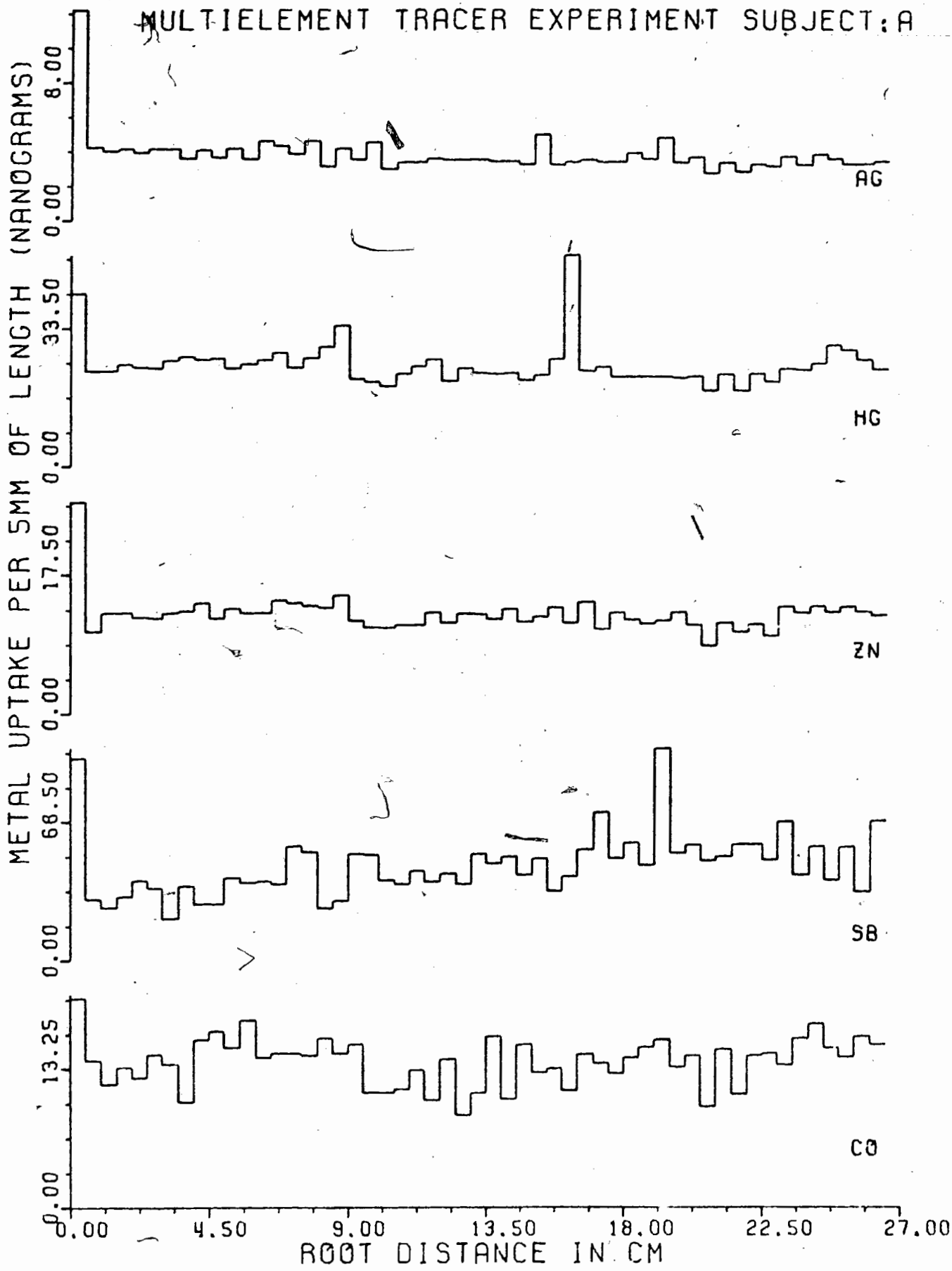

MULTIELEMENT TRACER EXPERIMENT SUBJECT: A
METAL UPTAKE PER SMM OF LENGTH (NANOGRAMS)
8.00
0.00
AG
33.50
0.00
HG
17.50
0.00
ZN
68.50
0.00
SB
13.25
0.00
CO
0.00
4.50
9.00
13.50
18.00
22.50
27.00
ROOT DISTANCE IN CM

FIGURE 42

particularly sharp increase of metal content along the length of the hair.

Furthermore, to check whether the duration of the soaking time may influence the shape of the patterns observed (say via diffusion or saturation effects) it seemed interesting to soak the hair samples for various lengths of time. Four hairs from subject F were soaked respectively for 30 minutes, 26 hours, 216 hours, and 216 hours again in the same multitracer solution. In Fig. 43 are shown the patterns obtained after 30 minutes of soaking in the solution. Due to the low activity of cobalt and antimony absorbed on the sample for such a short period of soaking, the patterns of these two elements were not determined to useful statistical precision and are not shown in the figure.

As before, in the case of hairs from subject A, the pattern for mercury is essentially flat from one end of the hair to the other, and the mass of mercury absorbed is the largest of the three elements measured. The silver and zinc patterns do exhibit (within the statistical limits available) some increase with distance down the hair shaft, but much less than seen in Fig. 2 for the patterns obtained with copper alone.

After 24 hours of soaking in the same solution, another hair from the sample subject taken at the same time shows (in Fig. 44) similar

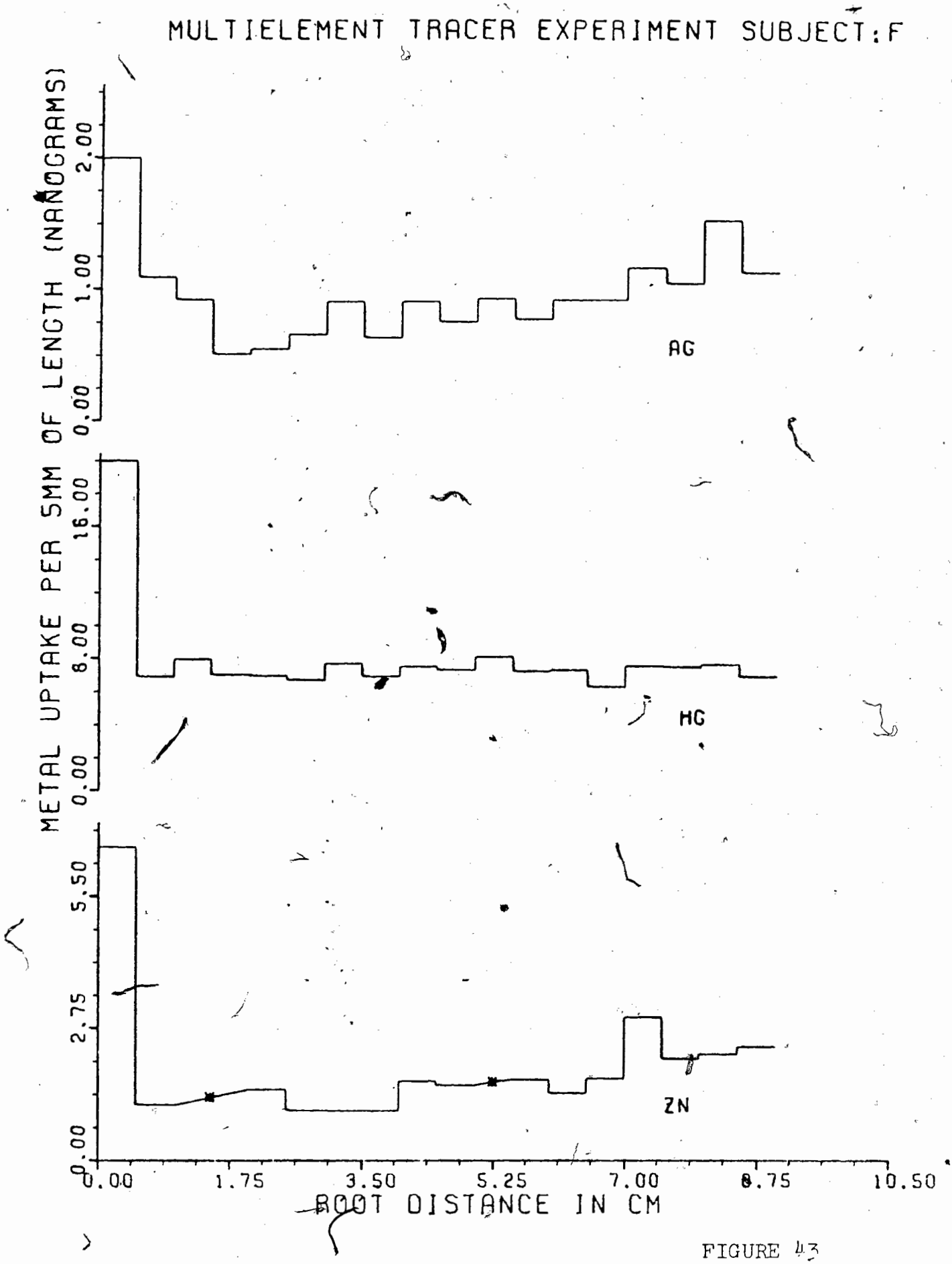

MULTIELEMENT TRACER EXPERIMENT SUBJECT:F
METAL UPTAKE PER SMM OF LENGTH (NANOGRAMS)
2.00
1.00
0.00
AG
16.00
8.00
0.00
HG
5.50
2.75
0.00
ZN
0.00
1.75
3.50
5.25
7.00
8.75
10.50
ROOT DISTANCE IN CM

FIGURE 43

MULTIELEMENT TRACER EXPERIMENT SUBJECT: F

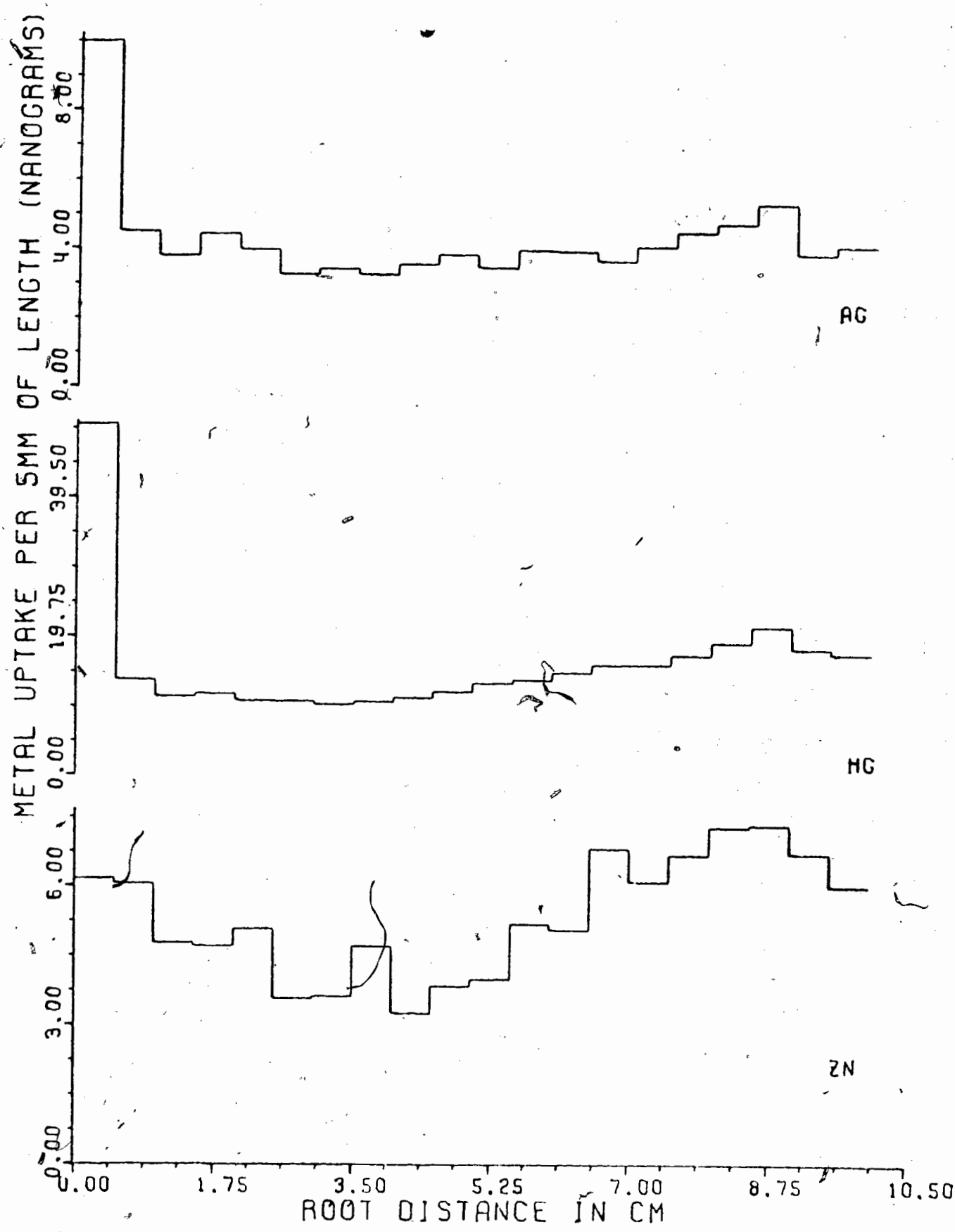


FIGURE 44

patterns to those in the previous figure; some small slope in the mercury pattern and some structure in the zinc pattern are now visible, but no evidence of pattern "saturation".

In view of such results, it was decided to use a very long time of soaking, with the additional expectation that cobalt and antimony might be detected in the hair fiber.

After 216 hours of soaking in the multi-element solution (Fig. 45 and 46), it is possible to measure the amount of each of the five elements absorbed in the two hair samples used in this experiment. It can be seen that the mercury and silver patterns are still flat. The zinc patterns are less structured than before, perhaps reflecting the competition from the increased mercury or silver absorbed. The patterns for cobalt and antimony, however, showed a broad minimum centred near 2 cm from the root, and rise to higher concentrations starting at 4 cm from the root.

It was somewhat surprising to find similar patterns for elements of such dissimilar chemical character. It was therefore decided to verify this result with hairs from another subject.

The very strong variation of the quantity of metal absorbed during the single tracer experiments along hairs which had been previously bleached suggested the use of hairs from such a subject in

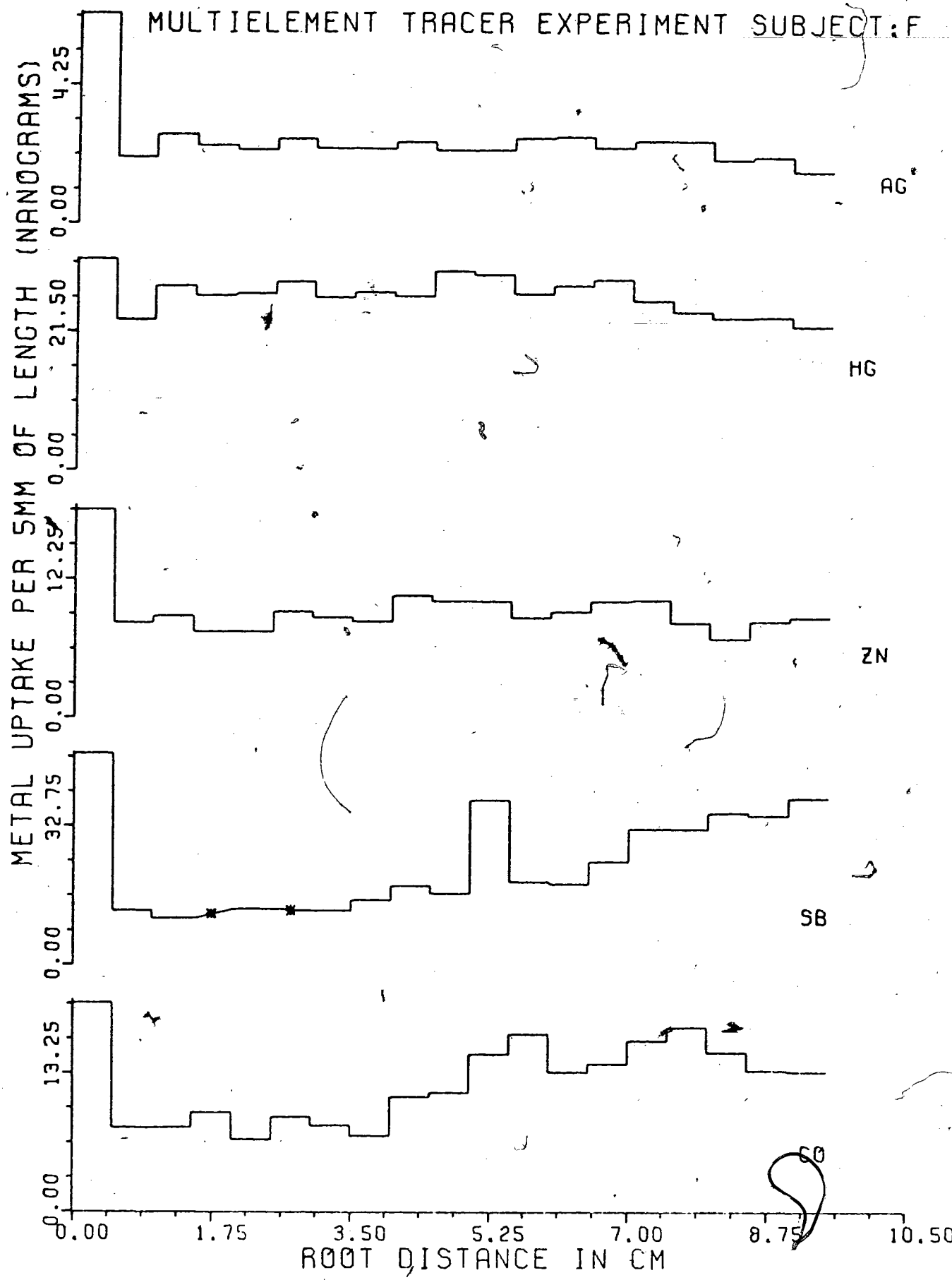

MULTIELEMENT TRACER EXPERIMENT SUBJECT:F
METAL UPTAKE PER 5MM OF LENGTH (NANOGRAMS)
4.25
0.00
AG
21.50
0.00
HG
12.25
0.00
ZN
32.75
0.00
SB
13.25
0.00
CO
0.00
1.75
3.50
5.25
7.00
8.75
10.50
ROOT DISTANCE IN CM

FIGURE 45

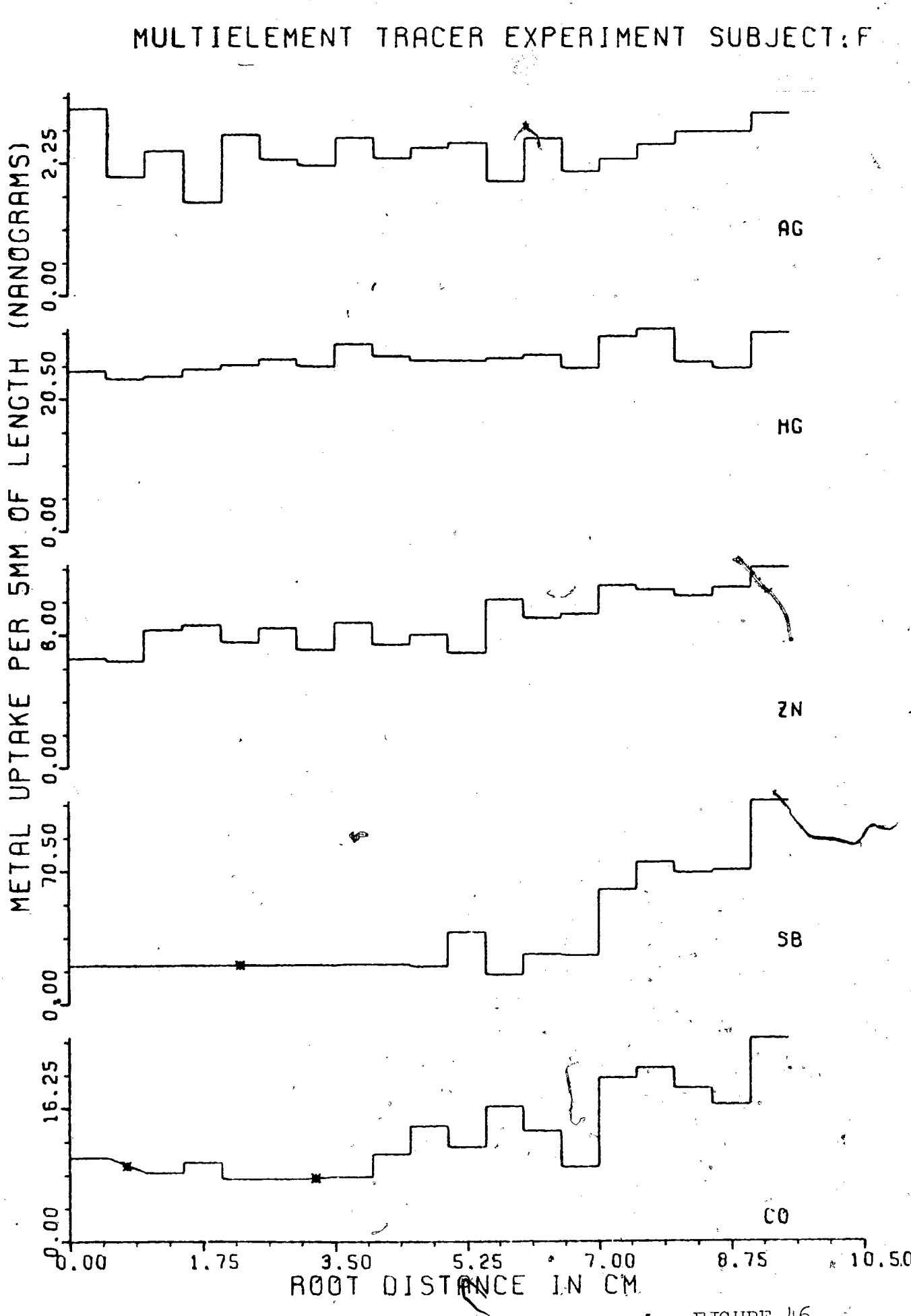

MULTIELEMENT TRACER EXPERIMENT SUBJECT: F
METAL UPTAKE PER 5MM OF LENGTH (NANOGRAMS)
2.25
0.00
AG
20.50
0.00
HG
8.00
0.00
ZN
70.50
0.00
SB
16.25
0.00
CO
0.00
1.75
3.50
5.25
7.00
8.75
10.50
ROOT DISTANCE IN CM

FIGURE 46

a multitracer experiment.

One hair from subject J was chosen for this experiment, exhibiting the characteristic bleached zones described earlier at roughly 4 cm to 11 cm from the root and from 13 cm to the distal end of the hair. The resulting patterns are shown in Fig. 47, where again the amount of silver, mercury, and zinc absorbed on each 5-mm section is constant along the hair length, throughout the bleached and unbleached regions. The pattern obtained for zinc, in particular, can be compared to the result of a single tracer zinc absorption experiment on the hair from the same subject (Fig. 30), where large variations in the amount of zinc absorbed were seen.

In contrast to the flat patterns obtained for mercury, silver, and zinc, the absorption patterns of cobalt and antimony in the same hair are both seen to exhibit sharply increased absorption in the same location, namely 4 to 11 cm from the root and perhaps from 13 cm to the distal end of the hair, regions where cobalt alone has also been shown to be absorbed preferentially (Fig. 34). Thus while the presence of other elements in the solution does apparently modify the patterns of zinc absorption into the hair structure, cobalt patterns are evidently less influenced by the presence of other elements since very similar patterns (although in reduced intensity) are obtained, whether this metal is alone or together with other elements in contact with the hair.

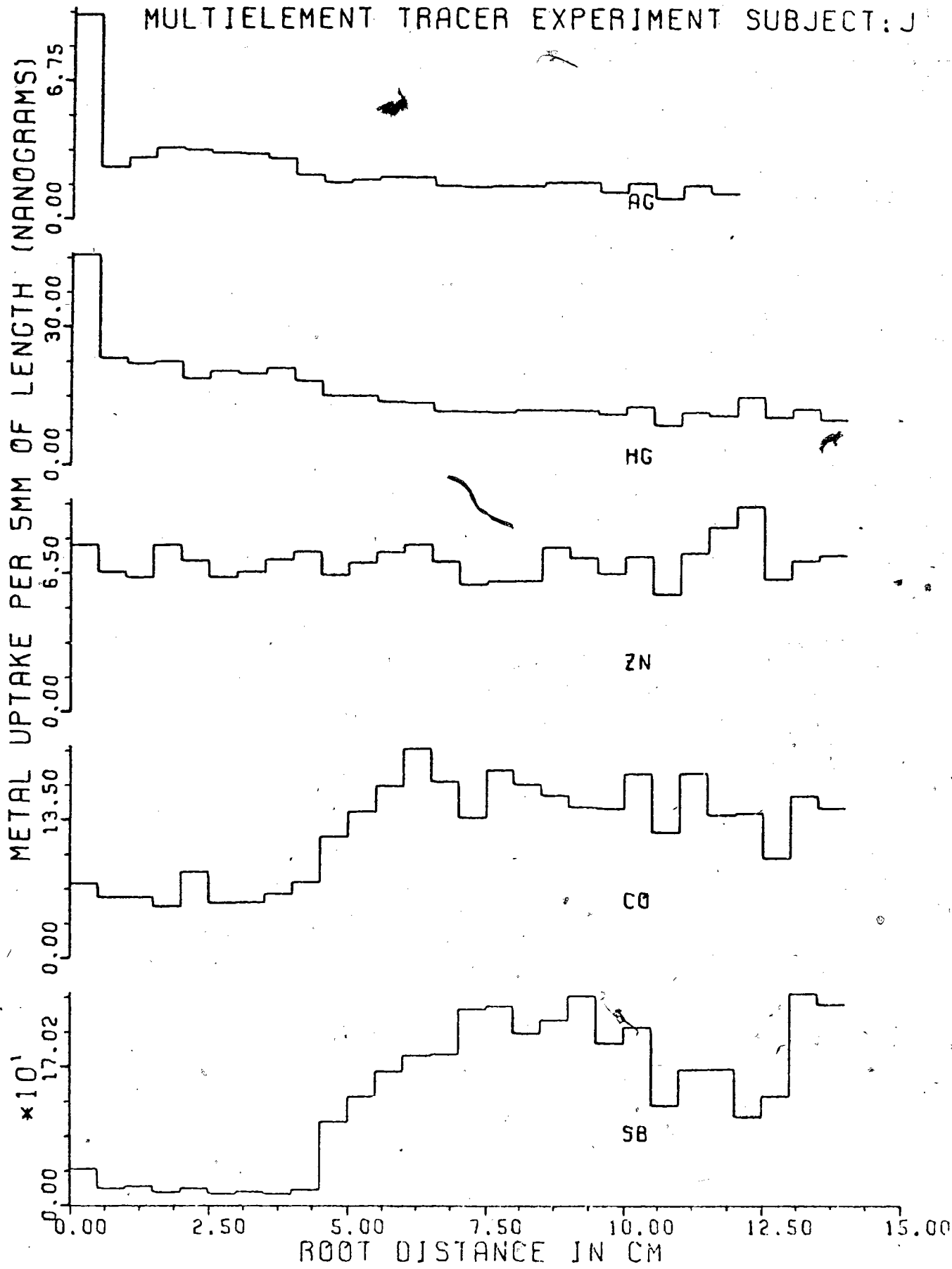

MULTIELEMENT TRACER EXPERIMENT SUBJECT: J
METAL UPTAKE PER 5MM OF LENGTH (NANOGRAMS)
AG
0.00
6.75
HG
0.00
30.00
ZN
0.00
6.50
CO
0.00
13.50
SB
*10¹
0.00
17.02
0.00
2.50
5.00
7.50
10.00
12.50
15.00
ROOT DISTANCE IN CM

FIGURE 47

Antimony, which was expected to behave like arsenic, and as such to be less strongly absorbed in the bleached region (see Fig. 40) is, on the contrary, characterized by an increased absorption there.

This behaviour could be explained if, at this pH value (4.5), antimony in solution exists as SbO^{+} or $Sb(OH)^{2+}$, which can be regarded as hydrolyzed forms of Sb^{3+}. Under such conditions, antimony may be preferentially attracted to regions known to absorb cations such as Cu^{2+} or Zn^{2+}. Arsenic, however, which in acidic solution exists as $HAsO_2$, is certainly expected to be less attracted to such zones.

It is difficult to understand why the absorption of zinc or silver does not increase with distance from the root in such regions, except in terms of a competition between these metals and mercury for the available binding sites in the keratin, since mercury has been shown to be absorbed readily in hair (Bat. 66b) and to influence the concentration pattern of copper as shown above.

From the data in Fig. 47 it appears that such competitive action is less important between mercury and cobalt (or antimony) in a highly heterogeneous medium such as the hair structure. A possible mechanism for such a difference in behaviour will be given later.

4) DISCUSSION

The results presented so far suggest the presence along the hair of sites where elements such as copper, zinc, cobalt, and arsenic (specific to a given region) are sorbed preferentially. The existence of these regions could be the origin of the substantial variations of natural metal content along the hair, reported in this work for copper, and by Smith (Smi. 64) for arsenic. Tracer experiments showed that the observed concentration patterns of these elements may have arisen entirely from the element added to the hair from external sources. These sources would not only include environmental contamination but also dietary intake, followed by excretion in sweat and sebaceous secretion with which the hair comes into contact.

The results obtained so far, especially during the copper ingestion experiment, do not contain evidence that dietary copper may enter the hair during the growth process occurring in the follicle itself.

The presence of such zones of preferential absorption along the hair may be interpreted as being due to:

- an increased number of "binding sites" available in this specific region of the hair which originate either from a chemical treatment such as bleaching, or from the natural

modification of the keratin composition due to denaturation with age.

- a deformation of the hair structure, which could have been caused by (for example) the oxidative cleavage by bleaching of the disulphide linkage in cystine (Fra. 72), shown to be partially responsible for the cohesion of the keratin (Spe. 47). Such a loosening of the hair structure might facilitate the diffusion of metallic ions, or even of bigger molecules, into and out of the hair structure.

It is reasonable to assume that absorption of trace element in hair could be in fact governed by a combination of the two processes previously described, since the oxidative cleavage of the cystine disulphide linkage results not only in a modification of the hair structure, but also in the formation of sulfonic acid groups (SO_3H) along the protein chains, which may be a possible site of binding for cations such as Cu^{2+}, Zn^{2+}, or Co^{2+}.

A similar mechanism may be speculated for unbleached natural hairs, where a large variation in the proportion of high sulfur proteins in the cortex has been demonstrated (Fra. 72). Because of the affinity of metals such as copper and zinc for sulphide groups, such variations should result in fluctuation of the amount of metal absorbed along the hair shaft.

It is difficult, then, to explain why, under such conditions, Zn and Cu are not absorbed preferentially on the same regions, except in the case of extreme modification of the hair structure (as produced by bleaching) when major uptake of zinc and copper has been observed in the same region of the hair.

The absorption of Zn and Cu in different regions of a hair may be rationalized in terms of a picture in which different regions have high concentrations of different functional groups such as -SH and CO_2H. If zinc becomes more easily bonded to regions of a specific character in the hair while copper becomes preferentially attracted to zones of a different character then the separate zones of Zn and Cu absorption are to be understood.

This hypothesis is supported by the observation that the zinc-sulfur bond is less stable than the copper-sulfur bond (Hin. 74) favouring a preferential binding of zinc to the negatively charged ionized carboxyl groups of the amino acids, rather than to ionized -SH sites.

The picture is also consistent with the observed increase of, e.g. Zn uptake, as the pH of the soaking solution is raised (i.e as the ionization of carboxyl groups becomes more complete). The very inhomogeneous structure of hair, however, does not allow a precise

identification of the entities these metals are bonded with, and it is likely that both sulfur and carboxyl groups are responsible to different extents for the binding of Cu and Zn in hairs.

The observation that bleached hair has a lower than normal zinc content, but at the same time preferentially absorbs zinc from solution, suggests that the loosening of the hair structure mentioned above is also responsible in part for the variation in the quantity of this metal and presumably others absorbed along the hair length.

The influence of the time of soaking on the amount of both zinc and copper absorbed in the hair is in any case a good indication that metal concentration is to some extent dependant on the rate of diffusion of these metals into the keratin.

The reason for the average amount of cobalt absorbed being smaller than that for zinc and copper in hairs taken on the same head and soaked in solutions of similar concentrations must be found in the weaker binding between this metal and sulfur and/or carboxyl groups. Furthermore, the fact that the amount of cobalt absorbed on hairs was found to be unrelated to the duration of the soaking may be explained by the limitation of the diffusion of this element to the outside region of the hair (cuticle). This phenomenon may have been due to a large extent to the solubility product of cobalt hydroxide being approached in the solution and at the pH used for the experiment, so

that much of the cobalt may have been present in colloidal form rather than as Co^{++}. This however was not verified experimentaly.

Such an interpretation may also explain the small increase of absorption observed in the vicinity of the bleached region of hairs, as compared to the sharp variation seen for Cu and Zn in indentical areas.

The concentration patterns of added arsenic in hairs, and especially for bleached samples, are to some extent similar to the patterns observed for natural zinc in bleached hairs. However, the presence of sharp maxima in the quantity of arsenic absorbed on some specific regions along single hairs is difficult to explain, except in terms of a hypothetical binding between H_3AsO_4 and an amino group of a protein chain.

Similarly, the competitive effect observed between Hg and Cu during the two-tracer absorption experiment, and between Hg, Zn, and Ag in the multi-tracer absorption experiment, may be rationalized in terms of the following hypotheses.

One region of the hair, say the high sulfur component of the cortex (matrix), contains most of the sites at which Hg, Cu, and Zn, and maybe Ag, are bound. However, because of a strong binding of mercury to sulfur, and since Zn and Cu seem to diffuse relatively

slowly into the hair structure, it may be speculated that most of the sulfur sites are occupied by mercury before the other metals reach the region where they would normally have been bound.

This supposition is supported by the observation that the amount of mercury absorbed is 4 to 8 times the amount of Zn taken up by the same hair, despite the fact that the multi-tracer solution was twice as concentrated in zinc as in mercury. On the other hand, the observation of the absence of such competitive action between mercury and cobalt (or antimony) would be difficult to interpret.

It may be, however, that cobalt (and possibly antimony) is preferentially bonded to sites closer to the surface of the hair (i.e. the cuticle) since no diffusion of Co into the hair on prolonged soaking was observed, in contrast with mercury which appears to saturate the interior sites of the hair.

Thus, since different regions are involved, competition between Hg and Co and Sb for the same sites does not occur to a significant extent.

Such a mechanism would not by itself explain the flat pattern of absorbed Hg which replaces the highly structured Zn patterns. If the Zn patterns are determined by a varying density of binding sites, the Hg patterns that replace them should be similarly structured. Perhaps

the mercury occupies not only the binding sites available to zinc (and as such interferes with absorption of this element) but may also be bonded to sites distributed uniformly in the inner region of the hair structure.

The results of Bate (Bat. 66b) on the comparative absorption of Zn and Hg seem to corroborate this hypothesis since the amount of Hg absorbed is, at pH 6.5, 11 times greater in mass and hence 2.1 times greater in the number of atoms than the quantity of Zn taken up by hairs, so that additional sites are evidently involved in Hg bonding.

CONCLUSION

The aim of the present work was not primarily to present a mechanism able to explain the binding of the few trace elements investigated here into the hair structure.

Although some hypotheses on such mechanisms have been presented, the raison d'etre of such work has been the delineation of the inorganic histopology of hair.

The existence of characteristic concentration patterns in individual hairs for both indigenous and added copper has been demonstrated, and the forensic applications of such features has been outlined. It remains a fact, however, that a method of identification or hair matching based on such pattern similarities is limited by:

- The necessity of identifying the hair samples to be matched as being both in the anagen period of the growth phase and as such, the necessity for such samples to have roots attached (since hair pattern features are displaced as hairs grow, and thus the relative growth "time scales" of two hairs to be matched must be known).

- The sometimes low correlation which exists between hairs coming from a single individual, whose trace element

patterns are flat and featureless.

- The possibility of "masking" a natural trace element pattern by hair treatments such as bleaching and/or dying.

It is evident that among all the elements investigated so far, copper patterns may be the most useful for identification purposes. This may be related to the strong binding of this element with sulfur, which may prevent a washing out of the pattern as appears to occur with zinc and maybe cobalt and arsenic.

The rather disappointing and featureless patterns obtained during the multi-tracer absorption experiment gives the impression that such method of "labelling" the characteristic absorption sites of an individual hair is not promising as a technique for hair matching. Such experiments, however, may be more successful for "finger-printing" the hair if mercury is replaced by an element whose absorption by the keratin will be less strong so that a complete saturation of most binding sites available (which appears to have occurred) will be avoided.

Appendix A

1) The Coefficient of Correlation

In order to determine whether the trace element concentration patterns measured on two hairs are similar to some degree of statistical significance, one may employ a test of linear correlation between the concentrations (say x and y) measured at a given distance from the root on each hair. The strength of linear relationship between two such variables may be measured by the "linear correlation coefficient (r).

The linear correlation between x and y is expressed through the relationship

$$y = a + bx$$

where the slope is given by

$$b = (N \sum_{1}^{n} x_i y_i - \sum_{1}^{n} x_i \sum_{1}^{n} y_i) / (N \sum_{1}^{n} x_i^2 - [\sum_{1}^{n} x_i]^2) \qquad \text{(Men. 71a)}$$

where N is the number of pairs of measurements (x_i, y_i) being compared.

If there is no correlation between the quantities x and y, then there will be no tendency for the value of y to increase or decrease with increasing x. Since we are looking for an interrelationship between the variables x and y, we can equally consider x as a function

of y, and see if the data correspond to a straight line of the form

$$x = a' + b'y$$

b' is similar to b and can be written as

$$b' = (N \sum x_i y_i - \sum x_i \sum y_i)/(N \sum y_i 2 - (\sum y_i)2)$$

If there is complete correlation between x and y, then there exists a relationship between the coefficients a, b and a', b'

$$y = -a'/(b') + (1/b')x = a + bx \Rightarrow a = -a'/(b')$$

$$\Rightarrow bb' = 1$$

If there is no correlation, both b and b' are null, since the variation of y is not proportional to a variation of x, and vice versa.

It is therefore possible to employ the experimental linear correlation coefficient

$$r = bb'$$

as a measure of the degree of linear correlation.

$$r = \frac{N \sum x_i y_i - \sum x_i \sum y_i}{\sqrt{[N \sum x_i 2 - (\sum x_i)2]\,[N \sum y_i 2 - (\sum y_i)2]}}$$

The value of r ranges from 0, when there is no correlation, to 1, when there is complete (or perfect) correlation.

2) Physical Interpretation of the Correlation Coefficient

The sample correlation coefficient, r, may be used as an estimate of a population correlation coefficient ρ, which would be obtained if the coefficient of correlation was calculated using all the points in the complete population. As such, r is a measure of common phenomena in the source of the two populations which generated the samples x and y.

For example, a coefficient of correlation equal to 0.5 will, in the case of two concentration distribution patterns, indicate that about 50% of the characteristics of the samples being compared have a common origin.

3) Application of Coefficient of Correlation Calculations to the Comparison Between Trace Element Concentration Patterns

Because of the large amount of data of which each concentration pattern consists, the calculation of a coefficient of correlation should yield a statistically significant measure of pattern similarity.

The calculations were performed on an IBM 370 computer on the different copper patterns reproduced in Fig. 2. Correlation was examined between the patterns in hairs taken from the same subject, and in hairs taken from different subjects, in order to determine if the former were significantly more correlated than the latter.

The significance of the difference between the two sets of correlation coefficients were tested by the student's t-test (Stu. 08). This test is designed to place a confidence interval on the significance of the difference between the means of small random samples drawn from two populations which possess a normal probability distribution. It is well adapted to the problem of calculating the probability of erroneously recognising two hair samples as originating from different subjects, when they in fact come from the same individual.

Assuming that the populations of correlation coefficients possess either a normal probability distribution, or, at least, cluster around some average value (Men. B71), one may calculate the numerical value of the test statistic t via

$$t = (R_1 - R_2)/(S\sqrt{(1/n_1) + (1/n_2)})$$

where R_1 is the mean of the correlation coefficients between concentration patterns of hairs taken from a single individual

R_2 is the mean of the correlation coefficients between concentration patterns of hairs taken from different individuals

S is the pooled estimator of the common population variance, and is equal to

$$S = \sqrt{(n_1-1)S_1^2 + (n_2-1)S_1^2 /(n_1 + n_2 - 2)}$$

n_1 and n_2 being the number of measurements made in each population of data.

S_1^2 and S_2^2 are respectively the estimators of the variances of tne two populations of correlation coefficients whose means are compared (Men. 71).

If the numerical value of the test statistic t is greater than the critical value t corresponding to a given degree of freedom (n_1+n_2-2), (Mer. 61), then it may be concluded that the mean of the correlation coefficients between concentration patterns of hairs taken from a single individual is greater than the mean of the correlation coefficients between concentration patterns of hairs sampled on different individuals, at the α level of significance.

If t is such that $t < t$, there is not sufficient evidence at the α level of significance to reject the hypothesis that the correlation coefficient means are equal, and it is therefore impossible to claim

that the concentration patterns of hairs coming from the same head are significantly more correlated than the patterns of hairs coming from different heads. The value of α can be chosen to produce the desired rigidity of the test.

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