have found that AChE and choline acetyltransferase (an exclusively neural enzyme in vertebrates) are present in constant ratio in whole adult *Drosophila*, in their brains, and in their thoracic ganglia; this is another indication that AChE in *Drosophila* is essentially restricted to the nervous system. Still, whether Kc-H cells are actually differentiating along neural lines must remain an open question since the highest AChE specific activity we have observed in these cells is only 1 percent of that in a *Drosophila* brain (*13–15*) and it is not known whether AChE at such low specific activities is a good marker for nervous tissue in *Drosophila*. Only by examining additional tissue-specific markers will it be possible to say whether Kc-H cells are neural elements or, indeed, whether they are faithful to any normal differentiative pathway. Nonetheless, the induction at physiological hormone concentrations of readily measurable amounts of a well-defined biochemical activity greatly enhances the utility of Kc-H cells for the study of ecdysone's action.

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12. In order to account for a 20-fold increase in AChE specific activity during the first 2 days of ecdysone treatment, the relative frequency of AChE-constitutive cells would have to increase 20-fold. Given the concurrent increase in the total population of 1.8-fold and the absence of cell death during this period, a 36-fold increase in the number of constitutive cells would be required. This cohort would have a mean division time of about 9 hours and, if it were sufficiently large, we should detect it in films. We have collected the pedigrees of more than 200 cells during β-ecdysone treatment. No intermitotic time less than 12 hours has been observed. From the absence of the rapidly dividing cells in our sample, we conclude that these cells, if present, represent ≤ 2.5 percent of the population initially. This being so, they should represent ≤ 50 percent of the population at 48 hours, and this heterogeneity would be detectable histochemically. The smaller the cohort of constitutive cells, the higher must be the activity of each cell and the more readily detectable in histochemical tests. We do not detect heterogeneity of nearly the magnitude required by the hypothesis.
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Proton-Induced X-ray Emission Analysis of Single Human Hair Roots

Abstract. *Collimated beams of 3.75 million electron volt protons were used to examine a 2-millimeter length of the root end of human hair; the concentrations of some hair root elements were correlated with the results of standard clinical assays of blood samples. The technique should be useful for the analysis of micro amounts of biological tissue.*

With the rapid development and application of proton-induced x-ray emission (PIXE) analysis, considerable interest has been generated in measurement of the elemental content of biological samples. Horowitz and Grodzins (*1*) presented an interesting example of PIXE analysis of elements along the length of a hair strand. Cookson and Pilling (*2*) reported elemental distributions determined by scanning a proton microbeam across the diameter of a single hair. Lazar (*3*) commented that many difficulties are encountered in obtaining valid correlations between the elemental content of a hair strand and medical factors, primarily be-

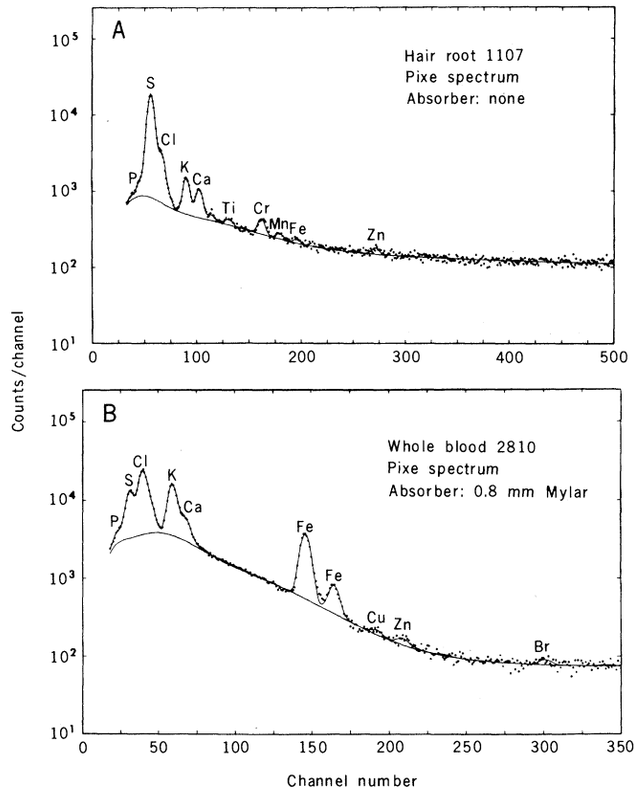

Fig. 1. Proton-induced x-ray emission spectra of (A) hair root and (B) whole blood samples. Values for Fe, Cu, Zn, and Br in 2 μl of blood are 642, 8.0, 11.0, and 7.2 ng, respectively. The blood spectrum is for the only child in whom copper was detected in both blood and hair root.

cause of externally adhered constituents. In this report we present results to show the feasibility of single hair root analysis by PIXE and the correlation between the elemental content of hair root and certain standard clinical assays of blood.

We recently pointed out (*4*) some of the advantages of hair root analysis over single-strand analysis; briefly, hair root analysis should reflect the most recent internal milieu and exclude externally adhered constituents such as those from shampoo and the atmosphere. Using the capability for elemental analysis of the Super FN Tandem Van de Graaff accelerator at Florida State University, we examined 2 mm (average dry mass estimated to be 9.34×10^{-6} g) of the root end of human hair. In a nutritional study involving 23 preschool children (*5*), we found correlations between the concentrations of elements within the hair root and correlations between the elemental concentrations and data from biochemical assays of blood.

Hair roots were mounted on plastic rings with each root end located at the center of a ring so that 2 mm of the end were bombarded. This method requires no backing for the sample and is described in more detail elsewhere (*4*). For whole blood samples, a 2-μl portion was deposited in the center of a 25-mm Millipore No. RHWP02500 ultrathin filter glued to a 25-mm plastic ring; this allowed the entire sample to be bombarded by the beam, which had a circular cross section 4 mm in diameter. Additional blood samples were sent to a commercial laboratory for a standard sequential automated (SA) analysis.

Proton beams of 20 na at 3.75 Mev were used to excite x-rays. A lithium-drifted silicon detector, 80 mm^2 in area and with a resolution of 155 ev at 5.9 kev, was used to measure x-ray spectra of hair root and blood samples at a distance of 6 cm from the sample. Spectra (Fig. 1) were subsequently analyzed by the completely automatic evaluation program REX. Fuller descriptions of the Florida State University system (*6*) and computer code (*7*) have been published.

Since hair roots differ in size within and between individuals, the ratio of the concentration of each mineral to that of sulfur was calculated, and these ratios were used in the correlation analysis. Minerals detected in all specimens were phosphorus, sulfur, chlorine, potassium, and calcium. Other elements detected in 30 to 56 percent of the specimens were manganese, silicon, zinc, and iron. Titanium was detected in three samples; copper and chromium were detected in one, although not in the same sample. Failure to detect an element indicates that under the conditions of the experiment, elemental concentrations within a particular sample matrix were below PIXE detection limits. For example, the detection limit for hair root zinc shown in Fig. 1 was 2.79 ng per 2-mm root end.

Several hair root elements tended to fluctuate in direct proportion to each other, as indicated by high correlations ($P < .01$) obtained for potassium and titanium with phosphorus; calcium, zinc, and manganese with chlorine; and manganese with calcium and zinc.

None of the whole blood elements detected by PIXE was correlated with the corresponding hair root element. Table 1 shows the hair root elements that were correlated with the SA data on blood; only for calcium was the concentration in serum correlated with the concentration in hair root, although several hair root elements were correlated with other elements in blood or with other biological criteria.

Table 1. Correlation of hair root element contents determined by PIXE analysis with analytical data on blood and with sex ($N = 23$). The data were analyzed by using the SPSS (Statistical Package for the Social Sciences) computer program; r is Pearson's product moment correlation coefficient.

Hair root element	Variable	r	$P <$
Silicon	Creatinine	.44	.017
Phosphorus	Alkaline phosphatase	.38	.036
	Carbon dioxide	.38	.037
	Serum sodium	.44	.018
Chlorine	Serum calcium	.36	.044
	Serum iron	.41	.026
	Serum glutamic pyruvic transaminase	.35	.048
	Triglycerides	.34	.054
	Hematocrit	−.44	.018
Potassium	Alkaline phosphatase	.37	.043
	Serum calcium	.34	.055
	Carbon dioxide	.41	.025
	Serum sodium	.38	.036
	Hemoglobin	.39	.035
	Hematocrit	.35	.049
Calcium	Serum calcium	.42	.024
Zinc	Serum iron	.39	.034
	Hematocrit	−.48	.011
Manganese	Serum calcium	.36	.044
	Triglycerides	.34	.054
	Hematocrit	−.35	.052
	Plasma ribonuclease	.38	.036
Iron	Serum chloride	−.44	.018
	Plasma ribonuclease	.35	.052
	Sex: male	.38	.036

Of particular interest was the fact that the hair root iron content was correlated with sex, being higher in males. All six of the hair root specimens in which iron was detected were from males. Sex differences in the iron content of hair strands of adults were noted by Perkons (*8*), but so far have not been reported in preschool children.

Of the entire group of children, only one child's hair root and blood showed detectable amounts of copper. (Figure 1B shows this subject's blood spectrum.) The concentrations of other elements in this child's hair root were of interest. For example, the sulfur, potassium, and phosphorus contents were considerably lower and the chlorine and zinc contents were higher than in the other children's hair roots.

Because correlations were found between the concentrations of hair root elements and certain standard biochemical assays of blood, further work with the hair root appears warranted to establish its suitability as a biological sample. At this time we do not know whether the hair root is sensitive enough to minor changes in the internal milieu or to the differences due to sex, age, or ethnicity of individuals or color of hair. The work reported here demonstrates the feasibility of analyzing very small samples quickly and inexpensively for several elements of medical interest. With additional study, it is conceivable that this technique could be applied to needle biopsies of other tissues.

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