Studies on Neutron Activation Analysis for the Measurement of Sodium in Nails As a Screening Test for Cystic Fibrosis*

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ABSTRACT

The present study points out the simplicity and ease of using fingernails and toenails to measure electrolyte levels as a test for cystic fibrosis. Procedures and various effects on the ranges of values for different categories of healthy and diseased subjects are discussed and the limitations of the method are indicated.

Introduction

The measurement of electrolyte levels in sweat is a reliable test for cystic fibrosis and is widely used. However, there are difficulties in collecting sweat specimens, especially on newborns, and investigations have been carried out using alternative biological materials for cystic fibrosis screening. Fingernails and toenails have provided promising results with elevated nail sodium levels being shown to correlate with the diagnosis of cystic fibrosis.1,2,3,7,8 The simplicity and ease of nail sample collection, storage, and transportation to the laboratory has led to the application of nail sodium determinations to mass screening of newborns for early detection of cystic fibrosis. Two large scale screening studies4,5,11 using automated neutron activation analysis have evaluated the use of nail analysis.

Several elements other than sodium have been shown to be elevated in the nails of cystic fibrosis patients and have been suggested as substitutes for sodium as diagnostic measurements. Leonard and Morris8 have noted the elevation of calcium and magnesium in the nails of fibrocystic children and have suggested that the sum total of sodium calcium and magnesium concentrations for a nail sample may be more useful than the sodium value alone. It has also been reported10 that levels of copper in the nails of fibrocystic patients are much higher than in normal individuals and a convenient flameless atomic absorption procedure has been developed.2

Surface contamination and washing procedures are important considerations in the
determination of any trace element in fingernails. Ideally, surface contamination should be completely removed without significantly depleting the trace element concentrations within the nail. Little agreement exists concerning what sort of washing procedure best accomplishes this task. Harrison and Tyree have reviewed a number of washing procedures and have documented the effects of their own method on the levels of several trace elements. They reported percentages of reductions in elements by washing, but were unable to ascertain whether or not this was due to removal of contamination or to leaching of the nails.

The present study was undertaken to investigate several washing procedures and to evaluate the effects of washing on the ranges of values obtained for various categories of diseased and healthy subjects. Sodium was measured because, as the most readily activated element in nails, it required far lower levels of activity per sample and as an ubiquitous element, it was considered a good measure of contamination.

Materials and Methods

Special Apparatus

Irradiation and Counting. Nail samples and standards were irradiated in the University of Florida Training Reactor (Gainesville, FL) or in the Georgia Institute of Technology Nuclear Sciences Reactor and were counted on a Dual Channel Auto Gamma System which contained a NaI(Tl) detector. A Model 9100B calculator was programmed for data reduction.

Sample Treatment. Fingernail washing was carried out on a large size reciprocating shaker. Nail washing vials were placed on the reciprocating base and held in place with adjustable rods. Nail samples were weighed on an electrobalance.

All glassware and plasticware were soaked overnight in Nochromix cleaning solution and rinsed in deionized water. Nail samples were washed in 25 ml snap-cap plastic vials and irradiated in polyethylene microcentrifuge tubes. Sodium standards were heat-sealed in capsules made from polypropylene test tubes. Nail samples and standards were counted in polystyrene screw-cap test tubes.

Standard Solutions. A stock solution containing 1000 µl per ml of sodium was prepared from high purity sodium carbonate and stored in a polyethylene bottle. Working solutions of 8.92, 17.18, 42.96, and 85.88 µl Na per ml were prepared from the stock solution.

Analytical Methods

Nails were clipped with clean clippers or scissors and were sent to the laboratory in small envelopes or plastic containers. The nails (2 to 25 µg) were scraped free of excessive dirt, weighed on the electrobalance, washed or not washed according to the study, dried and placed in polyethylene microcentrifuge tubes for irradiation. One quarter ml portions of standard solutions were heat-sealed in cleaned polypropylene capsules. Samples and standards were irradiated within a specific region of the reactor for the equivalent of one to two hours at a neutron flux of 10¹² cm⁻² sec⁻¹ and counted for four to 10 minutes on the Auto Gamma System.

In the washing and equilibration time study, the hands and feet of two children and two adults were soaked in three-liter beakers full of deionized water (one

* Model 1185, Nuclear Chicago, Des Plaines, IL 60016.
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beaker per each hand or foot) for 20 minutes. One hand and one foot of each subject had been dipped in sodium chloride solution and allowed to dry before being soaked, to ensure the presence of surface contamination. Nail samples were clipped from each hand and foot immediately before soaking, immediately after soaking and at one half hour, one hour, two hours, four hours, and five and one-half hours after soaking.

In vitro washing of nails was carried out in 25 ml deionized water contained in snap-cap plastic vials which were shaken gently on a mechanical shaker. Nails from two healthy adults, two healthy children (five and seven years), and two fibrocystic children (eight years and six months) were cut into small pieces and divided into five samples each. The first sample of each subject was not washed; the remaining four samples were washed for 5, 10, 15 and 30 minutes, respectively. Subsequent in vitro washing employed a 15 minute wash on the mechanical shaker.

Results and Discussion

The present investigation was carried out to determine the reliability of employing neutron activation analysis of nail samples as a screening test for cystic fibrosis. Irradiation of the samples was carried out in one of two Reactors and mailing samples for irradiation posed no problems. Counting was carried out on an automatic gamma counter such as is available in many laboratories for radioimmunoassay procedures.

The sodium content of nails reflects a combination of the sweat sodium level in the sweat glands under the nails and contamination from outside sources. Contamination is usually considerable, especially with children, and causes a great many false positives if nails are analyzed without any effort to reduce such contamination. If contamination can be removed, then the nail provides a crude but convenient means of estimating sweat sodium levels.

Woodruff et al. indicated that washing nails after clipping can leach out sodium causing false negative results when applied to cystic fibrosis screening and suggested that nails should be washed prior to clipping allowing a period of four hours for equilibrium to be reestablished between sweat and nail sodium levels.

The present investigation re-evaluated the neutron activation analytical method for nail sodium measurements providing an in depth study of the responses of nails to topical and in vitro washings.

Nail and Standard Linearity and Precision

Aliquots (0.25 ml) of the working standard solutions were irradiated and counted. Decay-corrected peak areas (total number of counts in the energy window minus the background) were plotted against concentrations to give a linear curve which passed through the origin.

As a measure of standard precision, several separately sealed and handled 0.25 ml samples of standard solution were irradiated together and counted. In addition, one standard sample was used as a comparison standard for calculation of the concentrations of sodium in the other samples.

As a measure of precision of nail analysis, 10 clippings, obtained from a healthy adult subject the morning after hands and nails had been thoroughly cleaned with soap and water, were analyzed separately. The results appear in table I with the standard precision data. The difference between the standard deviation of the analyses of several aliquots of standard solution of one concentration and that resulting from several counts of one standard solution aliquot gives an indication of the error present in encapsulation and post-irradiation transfer of the solutions. This error is less than the error present in analysis of
nail samples, the latter having problems of non-homogeneity, unremoved contamination and inherent counting geometry difficulties.

Washing and Equilibration Time Study

Previous investigators\textsuperscript{11} have asserted that washing nails in the laboratory tends to deplete the sodium in the nail to an unreliable level. It was suggested that nails washed while on the hand or foot, then clipped several hours later, would have contamination removed but would be at an equilibrium sodium level.

To test this hypothesis, the hands and feet of two children and two adults were soaked in deionized water with one hand and one foot of each subject having been dipped in sodium chloride solution and allowed to dry before being soaked, to ensure surface contamination. Nail samples were clipped from different nails from each hand and foot immediately before and at intervals after soaking. Feet were protected by conventional footwear whereas hands were not protected except for advising the subject to avoid contamination.

The results from one child and one adult are shown in figures 1 and 2, respectively. The other child and adult showed similar responses. The initial values on the children were much higher than the adults probably owing to considerably greater contamination before the sodium chloride treatment. The smaller changes in the adult values are ascribed to greater care taken by the adult in avoiding contamination during the study and to the normally lower levels of sodium in adult nails. It is noted that the adult's nails and the child's toenails remained at low sodium levels for several hours after washing while the child's fingernails increased markedly in

\textbf{TABLE I}

\textbf{PRECISION OF NAIL AND STANDARD SODIUM SOLUTION ANALYSES}

<table>
<thead>
<tr>
<th>Samples Analyzed</th>
<th>Number of Analyses</th>
<th>Sodium Concentration Mean ± S.D. (mEq per Kg)</th>
<th>Coefficient of Variation (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate standard solution samples</td>
<td>6</td>
<td>356 ± 38.3</td>
<td>10.7</td>
</tr>
<tr>
<td>One standard solution counted several times</td>
<td>5</td>
<td>370 ± 3.5</td>
<td>0.94</td>
</tr>
<tr>
<td>Ten nail clippings from one adult</td>
<td>10</td>
<td>93.2 ± 20.0</td>
<td>21.5</td>
</tr>
</tbody>
</table>

\textbf{Figure 1. Concentrations of sodium in a child's nails clipped before and at intervals after soaking.}
Figure 2. Concentrations of sodium in an adult's nails clipped before and at intervals after soaking.

Sodium content immediately after washing. This suggests that the high fingernail levels result from contamination and that the expected rise to an equilibrium level in sodium levels after washing does not occur. These findings in the subjects used in this investigation differ from those of Woodruff et al. They observed a more rapid rise in nail sodium levels following topical washing.

Laboratory Washing Study

Washing of nail clippings in the laboratory preserves the simplicity and advantages of nail sample collection. Therefore, the rates of contamination, removal and/or leaching of intrinsic sodium during washing of nail clippings from two healthy adults, two healthy children, and two fibrocystic children were studied. In figure 3 is shown the response to washing of one individual in each category. The normal child was five years and the fibrocystic child was eight years old. After the first five minutes of washing, sodium levels decreased gradually in the four children studied.

On the basis of the healthy children's and adults' responses, a 15-minute washing time was chosen for further work. It would appear from this study that the 15-minute wash would still leave fibrocystic children's nail sodium concentrations at a higher and distinguishable level from that of normal children. Sodium in the nails of the other fibrocystic infant (six months), however, was rapidly depleted to levels lower than those of the normal children. This is ascribed to the thinness of the infant's nails, and it was anticipated by the authors that if this method of analysis was ever to be used for cystic fibrosis screening, then separate washing parameters and normal ranges would have to be established for very young children.

Comparison of Washed and Unwashed Nails

Nail clippings were obtained from fibrocystic children, their siblings, their parents and healthy children. One sample from each subject was analyzed unwashed. Another sample from each subject was washed for 15 minutes in 25 ml of deionized water on the mechanical shaker. The results are listed in table II.

Unwashed nails demonstrated a wide separation in mean concentrations for healthy and fibrocystic children. They also showed a small and probably not significant separation between normal adults and parents of fibrocystic children. There was, however, considerable overlap between the ranges. A diagnostic test based on mea-
Measurement of sodium in unwashed fingernails would yield a large number of false positives if the lower limit of diseased values were placed low enough to minimize false negatives.

Washed fingernails from fibrocystic patients were surprisingly low in sodium, the mean being even lower than that of normal children's washed nails which was an unexpected finding in view of the fibrocystic child's response to washing shown in figure 3. This increased susceptibility to leaching was partly due to the younger age and thus thinner nails of some of the fibrocystic subjects; however, in some instances older fibrocystic children's nails were leached just as drastically. It was thought that the structure of nails from fibrocystic patients might be more susceptible to leaching than those from healthy individuals. Thus, a study was carried out where measurements were made of the leaching of sodium and calcium from nails of normals and children with cystic fibrosis. Measurements were made using a double beam double monochromator atomic absorption-emission spectrometer.* No difference was observed, however, between the leaching of sodium and calcium from nails of normals and children with cystic fibrosis.

It is evident, therefore, that the effect of in vitro washing of nail samples is extremely variable especially in children with cystic fibrosis.

**Conclusions**

The results of analyzing nail samples (unwashed and washed both before and after clipping) indicate the considerable problems in using nail analysis for screening for cystic fibrosis. Unwashed nails may

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**TABLE II**

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>Number of Subjects</th>
<th>Sodium Concentration (mEg per Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unwashed Nails</td>
</tr>
<tr>
<td>Fibrocystic children</td>
<td>15</td>
<td>218 ± 151</td>
</tr>
<tr>
<td>Healthy children</td>
<td>6</td>
<td>82 ± 42</td>
</tr>
<tr>
<td>Siblings of fibrocystic children</td>
<td>2</td>
<td>55 ± 63</td>
</tr>
<tr>
<td>Parents of fibrocystic children</td>
<td>15</td>
<td>54 ± 29</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>18</td>
<td>38 ± 24</td>
</tr>
</tbody>
</table>
differentiate between normals and fibrocystic patients but, because of contamination, there is a considerable overlap between the two categories. Washing after clipping appears to leach out most of the sodium for many of the fibrocystic individuals making this method for sample preparation useless. The approach of washing nails before clipping produced equivocal results. It was assumed from the work of Woodruff et al11 that the initial 20 minute wash would leach out most of the intrinsic sodium, remove contamination and that after about four hours the sodium level would have risen to equilibrium. This was not the case and values after five and one-half hours were roughly the same as those immediately after clipping. How well this topical washing would work to differentiate normals from abnormals would have to be evaluated using a large number of individuals in each category.

It appears to the authors that there are many variables in the measurement of nail sodium levels and that this test is of limited value in screening for cystic fibrosis.

References