Excretion of Copper in Sweat of Patients with Wilson's Disease During Sauna Bathing*

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ABSTRACT

Copper was measured by atomic absorption spectrometry in sweat samples obtained during sauna bathing (15 min at 93°) from seven patients (2♂, 5♀) with Wilson's disease (hepatolenticular degeneration). In one patient who was not being treated with penicillamine, the estimated excretion of copper in total body sweat during the sauna bath was 18 μg. In six patients who were receiving oral penicillamine (1 g per day), the estimated excretions of copper in total body sweat during the sauna bath averaged 106 μg, (range = 43 to 179). This study shows that sweating can be a significant route for the excretion of copper in patients with Wilson's disease who are being treated with penicillamine. Thermal induction of sweating by means of sauna bathing may possibly serve as a therapeutic adjunct to the customary treatment of Wilson's disease by means of low-copper diet and penicillamine.

Introduction

The presence of copper in sweat from healthy subjects has previously been reported by Mitchell and Hamilton,10 Consolazio et al4 and Hohnadel et al.7 In the present study, copper analyses have been performed upon sweat samples from seven patients with Wilson's disease (hepatolenticular degeneration), in order to ascertain whether or not thermally-induced sweating could serve as an adjunctive therapeutic

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TABLE I
Salient Clinical Data for the Patients with Wilson's Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>initials</th>
<th>Age at Initial Diagnosis (years)</th>
<th>Initial Sex and Age</th>
<th>Initial Symptoms and Findings</th>
<th>Kayser-Fleischer Corneal Rings at Time of Diagnosis</th>
<th>Duration of Therapy with Penicillamine</th>
<th>Condition at Time of Sweat Collection</th>
<th>Serum Ceruloplasmin* (mg/dl)</th>
<th>Serum Copper* (pg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.H. ♀ 18</td>
<td>12</td>
<td>Dysarthria, behavioral problems</td>
<td>+</td>
<td>6 years</td>
<td>Asymptomatic</td>
<td>2</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.D. ♂ 38</td>
<td>30</td>
<td>Tremor, dysarthria, risus sardonicus</td>
<td>+</td>
<td>4 years</td>
<td>Mild dysarthria</td>
<td>5</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.W. ♂ 25</td>
<td>18</td>
<td>None †</td>
<td>-</td>
<td>≥ 1 year</td>
<td>Asymptomatic</td>
<td>7</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.H. ♂ 18</td>
<td>17</td>
<td>Jaundice, hemolysis, hepatic cirrhosis</td>
<td>-</td>
<td>1 year</td>
<td>Asymptomatic</td>
<td>10</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.R. ♀ 26</td>
<td>24</td>
<td>None †</td>
<td>-</td>
<td>2 years</td>
<td>Asymptomatic</td>
<td>9</td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.S. ♂ 18</td>
<td>16</td>
<td>Tremor, dysarthria</td>
<td>+</td>
<td>2 years</td>
<td>Asymptomatic</td>
<td>1</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.M. ♂ 23</td>
<td>23</td>
<td>Incoordination, tremor, dysarthria</td>
<td>-</td>
<td>1 month</td>
<td>Mild dysarthria</td>
<td>11</td>
<td>71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Serum specimens which were obtained when the sweat was collected.
† Asymptomatic homozygous sibling detected during studies of the family of a patient with Wilson's disease.
≥ This patient had been treated with penicillamine for 2.5 years, but the penicillamine therapy had been discontinued more than six months before sweat collection (see text).

A method of eliminating copper from the body.

Materials and Methods

Patients

Three patients with Wilson's disease were studied at the University of Connecticut, and four patients with Wilson's disease were studied at the University of Wisconsin. The salient clinical data for the seven patients are summarized in table I. The patients were either asymptomatic or had very mild neurological signs at the time that sweat was collected. None of the patients had hyperbilirubinemia or severe abnormalities of liver function tests. The patients were all being treated with a low-copper diet, and six of the patients were receiving penicillamine** in an oral dosage of 1 g per day. The patient who was not receiving penicillamine had previously developed polymyositis as a complication of penicillamine therapy.14 The seven patients all gave their informed consent to the collection of sweat in a sauna bath, after the nature of the procedure had been fully explained. No adverse reactions to sauna bathing were observed in any of the patients.

Sweat Collection

Sweat samples were obtained by the arm-bag technique of Prasad et al12 with stringent precautions to minimize evaporative losses and copper contamination, as previously described.7 Sweat was collected during 15 min exposure to heat in a sauna bath at 93° with <5 percent relative humidity. Both arms were encased in polyeth-
COPPER EXCRETION IN SWEAT FROM PATIENTS WITH WILSON'S DISEASE

ylene disposable shoulder-length gloves†† which were secured with rubber bands at 3 cm below the axillae. Sweat samples were quantitatively removed from the gloves. The sweat which was collected from both arms was pooled and was centrifuged for 15 min at 900 × g in order to sediment dermal detritus. As reported in the previous study,7 the preliminary washing procedure was effective in removing copper from the surface of the skin, and there was no copper contamination from the polyethylene collection gloves. Venepuncture was performed immediately following the sauna bath, in order to obtain serum for analyses of copper and ceruloplasmin.

Analytical Methods

Analyses of copper in cell-free sweat and in serum were performed by the atomic absorption method of Sunderman and Roszel.17†† The normal range of copper concentrations in serum of healthy men was found by this method to average 119 (S.D. ± 19) μg per dl (N = 58).17 A typical recorder graph of analyses of copper in samples of sweat is shown in Figure 1. Analyses of ceruloplasmin in cell-free sweat and in serum were performed by the p-phenylenediamine oxidase method of Sunderman and Nomoto.16 The normal range of ceruloplasmin concentrations in sera of healthy men was found by this method to average 31.5 (S.D. ± 4.9) mg per dl (N = 29).16

Results

Measurements of copper in sweat from the seven patients with Wilson's disease are listed in table II. Also included in table II are the results of analyses of copper in sweat collected under identical conditions

†† Catalog No. BB-564, Bolab, Inc., Derry NH 03038.
†† Model 403 atomic absorption spectrometer, Perkin-Elmer Corp., Norwalk, CT 06856.

from 48 healthy adult controls (33 ♂, 15 ♀) as previously reported.7 No ceruloplasmin was detected in sweat from any of the patients, nor in sweat from seven healthy adult controls (4 ♂, 3 ♀). Estimations of the total excretion of copper in sweat from the entire body during 15 min in the sauna bath are given in table II, based upon the assumptions that: (1) the sweat collected from both upper extremities comprises an average of 11 percent of total body sweat21 and (2) the copper concentration in sweat obtained by the arm-bag technique is representative of the total body sweat.8 These assumptions should be viewed with some caution, since there are substantial individual variations in the regional distribution of sweating,6,13 and since the presence of a vapor-barrier (i.e.—the collection glove) might influence the concentrations of solutes in sweat.19
TABLE II
MEASUREMENTS OF COPPER IN SWEAT OF PATIENTS WITH WILSON'S DISEASE

<table>
<thead>
<tr>
<th>Patient (Initials, Sex and Age)</th>
<th>Sweat Collected from Both Arms (ml)</th>
<th>Sweat Copper (µg/dl)</th>
<th>Copper Excreted in Arm Sweat (µg)</th>
<th>Estimated Copper Excretion in Total Body Sweat* (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.H. ♀ 18</td>
<td>19</td>
<td>54</td>
<td>10.3</td>
<td>94</td>
</tr>
<tr>
<td>A.D. ♂ 38</td>
<td>48</td>
<td>41</td>
<td>19.7</td>
<td>179</td>
</tr>
<tr>
<td>Male Controls+ (N=33)</td>
<td>23±12</td>
<td>55±35</td>
<td>12.7±8.6</td>
<td>115±78</td>
</tr>
<tr>
<td>S.W. ♀ 25a</td>
<td>6</td>
<td>33</td>
<td>2.0</td>
<td>18</td>
</tr>
<tr>
<td>D.H. ♀ 18</td>
<td>10</td>
<td>175</td>
<td>17.5</td>
<td>159</td>
</tr>
<tr>
<td>V.R. ♀ 26</td>
<td>20</td>
<td>38</td>
<td>7.6</td>
<td>69</td>
</tr>
<tr>
<td>C.S. ♀ 18</td>
<td>18</td>
<td>26</td>
<td>4.7</td>
<td>43</td>
</tr>
<tr>
<td>G.M. ♀ 23</td>
<td>8</td>
<td>123</td>
<td>9.8</td>
<td>89</td>
</tr>
<tr>
<td>Female Controls+ (N=15)</td>
<td>6.8±3.3</td>
<td>148±61</td>
<td>10.1±3.4</td>
<td>88±31</td>
</tr>
</tbody>
</table>

*Assuming arm sweat = 11 percent of total body sweat.
+Previously reported by Hohnadel et al, 7
&This is the only patient who was not being treated with penicillamine at the time of the study.

Discussion

Consolazio et al4 performed copper balance studies upon three healthy men during prolonged (16 day) exposures to environmental heat. The men spent 7.5 hours each day in an environmental chamber which was maintained at 38° and 50 percent relative humidity. The subjects were sedentary, excepting for a daily period of 30 min of moderate activity on a bicycle ergometer. Copper balance was measured for three 4-day metabolic periods, following the initial 4-day period of acclimatization. During the 12 days of observation, the losses of copper in sweat from the three men averaged 1.6 mg per day, corresponding to 45 percent of the total daily intake. As a consequence of the losses of copper in sweat, the three men were each in negative copper balance, averaging —1.1 mg per day. Consolazio et al4 concluded that copper depletion might develop in healthy subjects who are exposed to environmental heat. Consistent with this observation, Szadkowski et al18 reported that the mean concentration of serum copper was significantly diminished in 55 workmen who were chronically exposed to extreme heat in a steel plant, in comparison to 27 control workmen who were not exposed to heat. Szadkowski et al18 attributed the thermally-induced hypocupremia to excretion of copper in sweat, although they did not actually perform any measurements of copper in sweat samples from the workmen.

The possibility that copper might be present in sweat primarily as a constituent of ceruloplasmin was investigated in the present study, inasmuch as Page and Remington11 and Jirka and Blanicky8 have previously detected minute amounts of ceruloplasmin in normal human sweat by use of immunochemical methods. Ceruloplasmin was not detected in sweat samples from patients with Wilson's disease or from healthy control subjects by the sensitive enzymatic procedure which was employed in this study. The observed concentrations...
of copper in sweat greatly exceed the quantities which could be attributed to traces of ceruloplasmin. Furthermore, studies which are in progress in our laboratory indicate that copper in pooled specimens of sweat from healthy subjects and from patients with Wilson's disease can be quantitatively recovered following ultrafiltration of sweat through cellulose membranes (Amicon PM-10) in a pressure dialysis apparatus.*

Only one patient (S.W. J 25) who was not receiving penicillamine therapy was available for study. This was the sole patient in whom diminished excretion of copper in sweat was observed. Investigations of copper excretion in sweat from additional patients with Wilson's disease who are not receiving treatment are needed in order to determine whether or not any abnormality in the elimination of copper in sweat is characteristic of Wilson's disease. In the six patients with Wilson's disease who were being treated with penicillamine, the excretions of copper in sweat during sauna bathing were within the ranges of values that were observed in healthy subjects. In the patients who were ingesting penicillamine, copper might conceivably have been present in sweat in the form of a penicillamine complex, since various other drugs (e.g. sulfonamides, amphetamines, quinine and methadone) have previously been shown to be excreted in sweat.2,5,15,19,20

Based upon the findings of the present study, asymptomatic patients with Wilson's disease who are being treated with penicillamine excrete approximately 100 µg of copper in total body sweat during a single 15 min period in a sauna bath. Metabolic balance studies are necessary to ascertain whether or not elimination of copper in sweat contributes to negative copper balance in these patients, since it is possible that increased loss of copper in sweat might be compensated by diminished excretion of copper in urine or feces. When suitable patients with Wilson's disease become available for study, the authors plan to measure the effect of thermally induced sweating upon copper balance, before and after the initiation of therapy with penicillamine.

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References