SOME BIOCHEMICAL CHANGES INDUCED BY CYANIDE INTOXICATION DURING SMOKE INHALATION

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ABSTRACT

Cyanide poisoning is a major factor that contributes to death due to smoke inhalation. The aim of the current study is to explore the postmortem stability of cyanide concentration in blood and tissues of fire victims and to evaluate the relation between glucose, lactate, anion gap and acute cyanide poisoning in smoke inhalation.

For this purpose 30 rats exposed to combustion products containing HCN for 1 hour were used as the animal model. Biochemical data were obtained by measuring cyanide (CN-) concentration, carboxyhemoglobin (COHb) saturation, glucose, lactate, and anion gap levels in dead rats and living ones before and after treatment as well as by measuring CN-, COHb and lactate in fire victims specimens.

Results showed a slight reduction in COHb saturation and a significant decrease in cyanide concentration in postmortem blood and tissues of rats during 24 hours to 3 days when left at 25-30ºC compared to zero time. There was a differential disposition of inhaled HCN, with the highest tissue levels found in the lung, brain, liver and spleen in decreasing order. Postmortem lactate (blood, lung, brain, and liver) of rats was elevated with respect to time and temperature with disappearance of glucose. From stability data, it was suggested that about 50% and 100% of the original blood cyanide disappeared during 24 hours and 3 days respectively, while about 14% remained in lung and brain at 3rd day. On the other hand, results revealed the close correlation between anion gap, lactic acidosis and whole blood cyanide levels in smoke inhalation-induced cyanide poisoning. The serial blood lactate measurements are useful in predicting survival in fire victims with severe smoke inhalation.

Keywords: Cyanide Poisoning - Smoke Inhalation - Postmortem Blood.

INTRODUCTION

The major immediate toxic threats in fire situations are carbon monoxide, a multitude of irritating organic chemicals in the smoke, oxygen depletion, and heat. During the past 50 years, synthetic polymers have been introduced in buildings in very large quantities. Many contain nitrogen or halogens, resulting in the release of hydrogen cyanide and organic acids in the smoke as additional toxic threats (Alarie, 2002).

Carbon monoxide (CO) is produced by incomplete combustion of carbon-containing compounds such as wood, coal, and gasoline. It has 250 times more affinity for hemoglobin than oxygen does and produces its toxic effects by displacing oxygen and decreasing the oxygen-carrying capacity of hemoglobin. CO also impairs activity of several intracellular enzymes by binding to cytochrome oxidase. These effects cause tissue hypoxia (Weiss and Lakshminarayan, 1994).

Hydrogen cyanide (HCN) is produced by burning high nitrogen content such as plastics, cotton, rayon, wool, polyurethane foam, nylon, rubber, papers and acrylonitrile glue found in laminates. Similarly, a number of plants contain cyanogenic glycosides, which release hydrogen cyanide when they are hydrolyzed or ingested (amygdalin, cassava roots and sorghum grass). HCN causes tissue asphyxia by inhibiting intracellular cytochrome oxidase a, activity, the final step in oxidative phosphorylation, thus preventing mitochondrial oxygen consumption. Cyanide poisoning also arrests the tricarboxylic acid cycle. Affected cells can only generate adenosine triphosphate via anaerobic metabolism, and lactic acidosis results from the anaerobic conversion of pyruvate to lactate (Maclennan, 1998).

The two gases (CO and HCN) are the major causes of combustion-related fatalities and their effects in fires are additive because both contribute to tissue hypoxia by different mechanisms. In residential fires, cyanide poisoning
Animals:

1- Experimental animals

Animals:

Forty adult (*Rattus norvegicus*) rats weighing 150-250 g were obtained from the Experimental Animal House, Helwan, Egypt. Animals were housed in separated screen bottom cages. They were maintained in a controlled environment under standard conditions of temperature and humidity. They were fed *ad libitum* on a standard laboratory pellets purchased from El-Nasr Lab. Chem. Co., Egypt. They were allowed free access to water. Animals were divided into two groups. The first group (exposed) included 30 rats (15 males and 15 females). The second group composed of 10 rats (5 males and 5 females) unexposed, were used as control.

**Inhalation Exposure:**

A static inhalation exposure chamber for a whole-body exposure (*Draize et al.*, 1959) was performed for acute inhalation toxicity (single exposure). It is 0.58 m³ airtight glass chamber with stainless steel frame containing a door for the entrance of experimental animals and a fume hood for the burning materials.

The first group was exposed 1 hr to combustion products of a fire smoke resulting from burning of 10.4 g of each of the materials listed below (*Montgomery et al.*, 1975) to imitate residential fires.

**Hydrogen cyanide generated by combustion:**

<table>
<thead>
<tr>
<th>Material</th>
<th>mg HCN / g material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>1.1</td>
</tr>
<tr>
<td>Wool</td>
<td>6.5</td>
</tr>
<tr>
<td>Cotton</td>
<td>0.13</td>
</tr>
<tr>
<td>Nylon</td>
<td>0.78</td>
</tr>
<tr>
<td>Polyurethane foam</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The combustion products of these materials yield HCN concentration of approximately 175 mg/m³ (157.5 ppm). The upper and lower 95% confidence limits of the LC₅₀ of HCN in rats are 144 and 175 mg/m³/hour (*Ballantyne*, 1984). LC₅₀ is the median concentration to which animals are exposed for a specified time that will kill 50% of the animals within a fixed period of time after exposure.

**Samples collection:**

Dead animals were dissected exactly after 2 hours of exposure (zero time); blood which was completely liquid drawn from the heart by a syringe in plastic tubes, and left for 72 hours at 25-30°C. After 24 hrs at 25-30°C, part of this blood was taken and preserved at 4°C in sodium fluoride/oxalate for the last 48 hrs. Blood samples were taken from the tubes through intervals at zero, 6, 12, 24, 36, 48, and 72 hours for the determination of carboxyhemoglobin (COHb) saturation, cyanide (CN⁻), and lactate concentrations. In addition glucose and anion gap were measured at 2,6,12 and 24 hours.

Lung lobes, liver, brain, and spleen were excised, homogenized and treated like blood samples. Analysis of cyanide and lactate concentrations were carried out at the same intervals.

Blood was taken from the heart of living rats after 2 hours of exposure (before treatment). Then, at 2, 6, 12 and 24 hours after treatment with cyanide antidote (2.7 mg sodium nitrite followed by 112.5 mg sodium thiosulfate)
USA. using Synchron CX5 autoanalyzer, Beckman Coulter, Inc.,- Blood glucose was determined by Trinder (1969)
measured.
- Carboxyhemoglobin (COHb) saturation was
determined using spectrophotometric method described by
Mayes (1993).
- Cyanide:
  Qualitative and semiquantitative determination of HCN
  in blood was carried out using Drager test-tube method
  (Dragerwerk AG, 2400 Lubeck, Germany), in which the
  change of the yellow colour of the reactive zone of the tube
to red indicates a positive reaction.
  Cyanide quantitation was performed using headspace
gas chromatography described by Mayes (1993).
  A gas chromatograph fitted with a 2 ml x 4.6 mm
  internal diameter column packed with Porapak Q 100/120
  mesh (Perkin Elmer, Beaconsfield HP) was used. Oven
temperature was 165°C; detector/injector temperature was
250°C; carrier gas helium flow was adjusted to 30 ml/
minute; nitrogen selective detection was used.
- Lactate was measured using the method described by
- Blood glucose was determined by Trinder (1969)
  enzymatic method.
- Anion gap:
  Routine measurement of electrolytes usually involves
  Na+, K+, Cl-, and HCO3-. These values may be used to
  approximate the anion gap (AG) = (Na+ +K+) - (Cl- + HCO3-)
  (Ehrmeyer et al., 1998). The measurement was carried out
  using Synchron CX5 autoanalyzer, Beckman Coulter, Inc.,
  USA.
4-Statistical analysis:
  Statistical analysis was performed using Student’s t- test.
  All the results were expressed as means± standard error.
P<0.05 was considered significant.

RESULTS AND DISCUSSION

After 1 hour exposure to smoke inhalation containing
HCN concentration approximately 175 mg/m3 /hour, 20
rats from the 30 exposed ones were died and 10 were
survived. The ratio of dead to alive was 2: 1. Of the dead 20
rats, 13 weighed 200-250 g and the other 7 rats weighed
150-200 g. The 10 survived were 6 females and 4 males
whereas the dead were 9 females and 11 males.

Results presented in Table (1) showed that cyanide
concentration in postmortem blood and tissues of rats
which measured by headspace gas chromatography
decreased significantly with respect to time and
temperature. Considering 2 hours of starting exposure as
the base line (zero time), cyanide concentration in blood,
lung, brain, liver, and spleen were reduced to 56.5%,
56.0%, 47.2%, 46.05%, and 35.48% respectively within 24
hours at 25-30º C. In samples left for 72 hours at the same
temperature, cyanide disappeared almost completely from
blood, liver, and spleen, while about 14% of the original
amount of cyanide remained in lung and brain. But
samples preserved after 24 hours at 4º C till the end of 72
hours indicated that cyanide remained in all examined
tissues (blood 35%, lung 32%, brain 44%, liver 48%,
and spleen 49.6%). The results also revealed that blood
carboxyhemoglobin (COHb) saturation was slightly
reduced with time and temperature compared to zero time
(2 hours postmortem) and significantly increased compared
to unexposed controls.

Data in Table (2) showed the disappearance of
postmortem blood glucose completely after 72 hours and
the elevation of blood and tissue lactate (lung, brain, liver)
with respect to time and temperature. At zero hour lactate
concentration was higher in all tissues examined by 94.4%
to 324.0% compared to the control kept at the same
temperature (25-30º C). At 72 hours lactate levels were
increased significantly by 45.8% in blood, 94.2% in brain,
41.8% in lung, and 74.9% in liver with respect to zero time.
There was no significant difference between lactate
concentration in various tissues at 48 and 72 hours at room
temperature. But in storage at 4º C the stability of lactate
stayed for 2 days.

Table (3a) showed that in dead animals a significant
decrease of Na+, Cl-, HCO3-, and an increase of K+ with
respect to postmortem intervals comparing to control rats.

Table (3b) indicated significant increase in K+ ion
concentration and anion gap accompanied by significant
decrease in HCO3- in living animals before treatment
comparing to control ones, but after treatment the anion
gap was corrected through 12 hours with the return of K+
and HCO3- to their normal levels.

Data in Table (4) revealed significant reduction of
COHb saturation and cyanide concentration to the normal
levels with the correction of lactic acidosis accompanied by
lowering blood glucose to normal level through 24 hours
Table (1): Effect of temperature on postmortem cyanide concentrations and COHb saturation in blood and tissues of rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature</th>
<th>Blood % COHb</th>
<th>Blood cyanide mg/L</th>
<th>Tissue cyanide mg/kg wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMI hours</td>
<td></td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>Brain X±S.E</td>
</tr>
<tr>
<td>Zero(2hrs)</td>
<td>25-30°C</td>
<td>15.0±1.0</td>
<td>4.6±0.9</td>
<td>3.6±0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0±1.0</td>
<td>3.6±0.8</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.7±0.9</td>
<td>3.3±0.9 *</td>
<td>2.8±0.05*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.5±1.1</td>
<td>2.0±0.1 **</td>
<td>1.9±0.01**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.0±1.1**</td>
<td>1.0±0.01**</td>
<td>1.4±0.01**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.5±1.2**</td>
<td>0.5±0.01**</td>
<td>1.0±0.01**</td>
</tr>
<tr>
<td></td>
<td>4°C</td>
<td>14.5±1.1</td>
<td>2.00±0.1</td>
<td>1.9±0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.5±1.1</td>
<td>1.8±0.01**</td>
<td>1.7±0.01**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.2±1.0</td>
<td>1.67±0.01**</td>
<td>1.4±0.01**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.0±0.8</td>
<td>1.46±0.1**</td>
<td>1.2±0.01**</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.0±0.0</td>
<td>0.12±0.01</td>
<td>0.4±0.01</td>
</tr>
</tbody>
</table>

Control=Unexposed rats.  ND=Not detected.  PMI=Postmortem intervals

*=Significant (p<0.05).  **= Highly significant (p<0.01).

Table (2): Effect of temperature on postmortem glucose, and lactate in blood and tissues of rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature</th>
<th>Blood glucose mg/dl</th>
<th>Lactate mg% (mg/dl or/100g wet tissue)</th>
<th>Blood X±S.E</th>
<th>Brain X±S.E</th>
<th>Lung X±S.E</th>
<th>Liver X±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMI hours</td>
<td></td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>X±S.E</td>
</tr>
<tr>
<td>Zero(2hrs)</td>
<td>25-30°C</td>
<td>240.0±2.9</td>
<td>120.0±1.5</td>
<td>79.3±1.4</td>
<td>110±2.0</td>
<td>89.2±1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>165.5±1.3**</td>
<td>132.5±0.9**</td>
<td>95.0±1.8*</td>
<td>121±1.8**</td>
<td>110.0±1.4**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>58.0±1.1**</td>
<td>144.3±1.5**</td>
<td>125.0±2.0**</td>
<td>136±1.0**</td>
<td>133.0±1.1**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.3±0.9**</td>
<td>168.9±1.8*</td>
<td>144.0±1.8**</td>
<td>150±2.0**</td>
<td>148.0±2.0**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0±0.5</td>
<td>175.0±1.6**</td>
<td>153.0±1.1**</td>
<td>155±1.9**</td>
<td>155.0±1.9**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ND</td>
<td>175.5±1.1**</td>
<td>154.0±1.0**</td>
<td>156±1.0**</td>
<td>156.0±1.6**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>215±2.0</td>
<td>28.3±1.4</td>
<td>40.8±1.1</td>
<td>33±1.2</td>
<td>39.0±1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4°C</td>
<td>15.3±0.9</td>
<td>168.9±1.8</td>
<td>144.0±1.8</td>
<td>150±2.0</td>
<td>148.0±2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.0±1.0**</td>
<td>170.0±1.1**</td>
<td>144.3±1.2**</td>
<td>150±1.0**</td>
<td>148.6±1.1**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ND</td>
<td>175.0±1.0**</td>
<td>145.0±1.1**</td>
<td>151±1.3**</td>
<td>149.0±1.2**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>220.0±3.3</td>
<td>7.0±1.1</td>
<td>15.6±1.5</td>
<td>19.0±1.2</td>
<td>22.0±1.9</td>
<td></td>
</tr>
</tbody>
</table>

Control=Unexposed rats.  ND=Not detected.  PMI=Postmortem intervals

*=Significant (p<0.05).  **= Highly significant (p<0.01).

Table (3a): Values of blood electrolytes and anion gap in dead rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>K+ mmol/L</th>
<th>Na+ mmol/L</th>
<th>Cl- mmol/L</th>
<th>HCO3- mmol/L</th>
<th>Anion gap mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>X±S.E</td>
<td>X±S.E</td>
</tr>
<tr>
<td>Control</td>
<td>4.6±0.11</td>
<td>142±2.0</td>
<td>104.3±1.4</td>
<td>27.5±2.0</td>
<td>13.6±1.5</td>
</tr>
<tr>
<td>2 hr.</td>
<td>6.0±1.4</td>
<td>142±1.1</td>
<td>108±2.0</td>
<td>10.9±0.9**</td>
<td>30±1.2**</td>
</tr>
<tr>
<td>6 hr.</td>
<td>10.9±2.0**</td>
<td>129±1.2**</td>
<td>110±2.1**</td>
<td>9±1.0**</td>
<td>18.7±0.22**</td>
</tr>
<tr>
<td>12 hr.</td>
<td>17.6±1.9**</td>
<td>103±1.4**</td>
<td>101±1.9**</td>
<td>6.4±0.1**</td>
<td>13.8±0.2</td>
</tr>
<tr>
<td>24 hr.</td>
<td>24.9±1.7**</td>
<td>86±1.8***</td>
<td>84±1.8***</td>
<td>5.6±0.1**</td>
<td>11.2±0.1*</td>
</tr>
</tbody>
</table>

Control=Unexposed rats.  *=Significant (p<0.05).  **= Highly significant (p<0.01).
Table (3b): Values of blood electrolytes and anion gap in survived rats before and after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time</th>
<th>K⁺ mmol/L</th>
<th>Na⁺ mmol/L</th>
<th>Cl⁻ mmol/L</th>
<th>HCO₃⁻ mmol/L</th>
<th>Anion gap mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>4.6±0.11</td>
<td>142±2.0</td>
<td>104.3±1.4</td>
<td>27.5±2.0</td>
<td>13.6±1.5</td>
</tr>
<tr>
<td>B. T.</td>
<td></td>
<td>6.5±0.3**</td>
<td>141±1.2</td>
<td>104±2.2</td>
<td>12.0±0.9**</td>
<td>31.5±1.0**</td>
</tr>
<tr>
<td>2 hr. A.T</td>
<td></td>
<td>5.5±0.2**</td>
<td>140±1.1</td>
<td>103±2.0</td>
<td>19.5±0.9**</td>
<td>23.0±1.0**</td>
</tr>
<tr>
<td>6 hr. A.T</td>
<td></td>
<td>5.0±0.1*</td>
<td>141±2.1</td>
<td>105±1.8</td>
<td>25.0±0.9</td>
<td>18.0±0.5*</td>
</tr>
<tr>
<td>12 hr. A.T</td>
<td></td>
<td>4.5±0.11</td>
<td>140±1.8</td>
<td>105±1.9</td>
<td>27.0±0.7</td>
<td>12.5±0.3</td>
</tr>
<tr>
<td>24 hr. A.T</td>
<td></td>
<td>4.5±0.1</td>
<td>141±1.4</td>
<td>103±1.8</td>
<td>29.0±0.7</td>
<td>13.5±0.3</td>
</tr>
</tbody>
</table>

Control = Unexposed rats. B.T. = Before treatment. A.T. = After treatment. * = Significant (p<0.05). ** = Highly significant (p<0.01 or 0.001).

Table (4): Values of COHb saturation, cyanide, lactate and glucose concentrations in survived rats before and after treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%COHb</th>
<th>Cyanide mg/L</th>
<th>Lactate mg/dl</th>
<th>Glucose mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hr.</td>
<td>24.5±1.2*</td>
<td>10±1.0*</td>
<td>2.6±0.1**</td>
<td>2.0±0.1**</td>
</tr>
<tr>
<td>6 hr.</td>
<td>-</td>
<td>8±0.9**</td>
<td>-</td>
<td>1.7±0.01**</td>
</tr>
<tr>
<td>12 hr.</td>
<td>-</td>
<td>6±0.8*</td>
<td>-</td>
<td>1.0±0.01*</td>
</tr>
<tr>
<td>24 hr.</td>
<td>-</td>
<td>5.6±0.1</td>
<td>-</td>
<td>0.4±0.01</td>
</tr>
<tr>
<td>Control</td>
<td>5.0±0.1</td>
<td>0.12±0.1</td>
<td>7.0±1.1</td>
<td>220±3.3</td>
</tr>
</tbody>
</table>

Control = Unexposed rats. A.T. = After treatment. B.T. = Before treatment. * = Significant (p<0.05). ** = Highly significant (p<0.01).

Table (5): Human postmortem values of COHb saturation, cyanide and lactate concentrations in fire victims.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Postmortem intervals (hrs)</th>
<th>% COHb X±S.E</th>
<th>Cyanide mg/L or/kg wet tissue X±S.E</th>
<th>Lactate mg/dl or/ 100g wet tissue X±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Control</td>
<td>10±1.2</td>
<td>0.13±0.01</td>
<td>30.0±3.3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>28.4±1.2**</td>
<td>5.3±0.5</td>
<td>136.0±2.1**</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>25.9±1.1**</td>
<td>4.0±0.3</td>
<td>148.0±1.8**</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>30.2±2.0**</td>
<td>2.4±0.1**</td>
<td>172.0±1.4**</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>34.8±1.2**</td>
<td>1.12±0.01</td>
<td>185.0±1.9**</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.5±0.03</td>
<td>32.2±1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>--</td>
<td>8.3±0.3</td>
<td>125.4±1.6**</td>
</tr>
<tr>
<td></td>
<td>12</td>
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<td>6.5±0.12</td>
<td>142.6±1.4**</td>
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<td>24</td>
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<td>2.6±0.03</td>
<td>160.0±2.2**</td>
</tr>
<tr>
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<td>48</td>
<td>--</td>
<td>2.3±0.03</td>
<td>180.5±2.1**</td>
</tr>
<tr>
<td>Brain</td>
<td>Control</td>
<td>0.5±0.03</td>
<td>20.0±1.1</td>
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<td>4.2±0.11</td>
<td>99.5±0.96**</td>
</tr>
<tr>
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<td>3.2±0.11</td>
<td>145.6±1.1**</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>--</td>
<td>1.9±0.01</td>
<td>163.3±2.0**</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>--</td>
<td>1.2±0.02</td>
<td>177.6±1.5**</td>
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<tr>
<td>Liver</td>
<td>Control</td>
<td>0.5±0.03</td>
<td>27.0±1.2</td>
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<td>3.0±0.12</td>
<td>126.9±1.2**</td>
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<tr>
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<td>2.2±0.06</td>
<td>150.8±1.0**</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>--</td>
<td>1.5±0.01</td>
<td>166.5±1.8**</td>
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<tr>
<td></td>
<td>48</td>
<td>--</td>
<td>0.8±0.01</td>
<td>182.6±1.6**</td>
</tr>
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</table>

Control: Medicologal samples of individuals whose injuries were not caused by fire. *=Significant (p<0.05). **= Highly significant (p<0.01).
after combined treatment of cyanide poisoning with sodium nitrite and sodium thiosulfate (cyanide antidote).

Table (5) showed that in fire victims, cyanide concentration which measured by headspace gas chromatography was very site dependent and significantly differs from controls except at 48 hours in postmortem blood. COHb saturation ranged from 25.9%– 35.5%. Lactate concentration significantly increased with respect to different time intervals in blood and tissues.

At the same time blood cyanide concentration in animal and human samples were also measured by Drager test-tube method as a primitive test. The low detection limit of this test was 0.5 mg CN/L.

In the present study 30 rats exposed to combustion products containing HCN were observed and their response reactions distinguished to 3 stages. Stage one was characterized by restlessness, increasing activity and violent attempts to leave the chamber. Stage two involved a decrease in voluntary muscular activity followed by collapse to the floor of the exposure chamber and convulsive movements of the tail with respiration varying from very deep, slow, and regular to irregular respiration. In stage three, there was no movement at all other than respiration which diminished in both amplitude and frequency until death.

Results revealed 20 deaths in which aged animals comprised 13 of them and young ones comprised 7. The ten rats that survived exposure were 6 females and 4 males which may be attributed to sex differences in susceptibility.

From the present results a slight reduction in COHb saturation and a significant decrease in cyanide concentration were observed in postmortem blood and tissues of rats during 24 hours to 3 days when left at 25-30°C. Cyanide was fairly stable when stored at 4ºC but tends to decrease slowly as temperature increases (Chikasue et al., 1988; Mortya and Hashimoto, 2000), whereas COHb behaves similarly at 4ºC but tends to decrease slowly as temperature increases (Fechner and Gee, 1989; Kunsman et al., 2000).

At the same time postmortem lactate (blood, lung, brain, and liver) of rats elevated with respect to time and temperature without significant difference between various tissues (lung, brain and liver) studied at 2nd and 3rd days. These results are in accordance with Iannuzzi et al. (1988; 1989) who reported increased concentration of postmortem lactate in an uncontrolled accident situation.

Glucose concentration in postmortem blood samples is of a little value unless it is high. Glycolysis continues after death and blood glucose falls extremely rapidly (Forrest, 1993). This is in agreement with the current results (Table 2).

The ability of cells to maintain the concentration gradient of electrolytes between the intra- and extra- cellular spaces is energy dependent. Once death has taken place, these concentration gradients can no longer be maintained and there is a rapid flux of the smaller molecules down the appropriate concentration gradients. The most immediate effect is a very rapid rise in serum potassium, thus determinations in postmortem samples are of little or no probative value. But serum sodium and chloride concentrations tend to decrease after death more slowly (Wilkie and Bellamy, 1982; Forrest, 1993; Imaeda, 1999).

The decrease of HCO₃⁻ was reported by Yamamoto and Yamamoto (1978) who observed significant positive correlation between postmortem cyanide and PCO₂ values. These findings are in consistence with the present results, and are confirmed by Karkela (1993) who reported the same changes in CSF after death where glucose, pyruvate, Na⁺, and Cl⁻ levels declined, while lactate and K⁺ increased over time after death.

An elevated COHb levels in fire victims may be considered an index of cyanide poisoning because of the significant correlation between measured levels of both toxins (Clark et al., 1981; Matushara et al., 1990; Baud et al., 1991). To the contrary, postmortem studies did not support this correlation. COHb alone is a poor predictor of the severity of toxicity, because a low or non detectable level does not exclude the possibility of inhalation injury (Sokal and Kralkowska, 1985; Lundquist et al., 1989). Baud et al. (1991) reported that 55% of patients who died from smoke inhalation had levels of both cyanide and COHb below those considered potentially lethal. The present results are in agreement with the previous studies.

To obtain basic data on the postmortem stability of blood and tissue cyanide in corpses, the aforementioned experimental animal design was conducted to reproduce circumstances similar to the fire victims. Bariotto et al. (1994) and Wardaszka et al. (2005) reported that cyanide can be produced and also degraded in blood and tissues, making interpretation of blood levels difficult. However, the designed animal model may be useful for estimating how much cyanide disappears from fire victims' blood and tissues between death and sampling. From the results of stability data, it was suggested that about 50% and 100% of the original blood cyanide disappeared during 24 hrs and 3 days respectively, while about 14% remained in lung and brain at 3 days. On the other hand, the postmortem correlation between cyanide and lactate concentration in various tissues examined are not useful as long-term indicator of cyanide poisoning. Although significant metabolic perturbations are present at the time of death, but differences do not persist up to 48 hours postmortem.

Animal study results show a differential disposition of inhaled HCN, with the highest tissue levels found in the lung, brain, liver and spleen in decreasing order. These data seem to collaborate the evidence from other animal studies and from clinical reports that emphasize the importance of these organs in cyanide toxicity (Ballantyne et al., 1972;
Ballantyne, 1987; Zhang et al., 2005). In addition, tissue samples may be especially valuable if death was due to inhalation of lipophilic compounds. As HCN is moderately lipid soluble, blood concentrations should be low, the other tissues are the most useful specimens especially brain (Knight, 1996).

The current results indicated that the major metabolic abnormality was lactic acidosis which can be corrected over 24 hours after combined treatment with sodium nitrite and sodium thiosulfate (cyanide antidote). Many studies confirmed the positive correlation between the severity of cyanide poisoning and high lactate concentrations (Graham, 1977; Singh, 1989; Baud et al., 1991, 1996, 2002; Borron and Baud, 1996), as well as carbon monoxide concentration (Sokal and Kralkowska, 1985). However, Baud et al. (1991) reported that plasma lactate concentrations at the time of admission correlated more closely with blood cyanide than with blood carbon monoxide concentrations. Benaissa et al. (2003) confirmed that plasma lactate is mildly elevated in pure CO-poisoning.

Dreykluft et al. (2004) and Shusterman and Hargis (1994) drew attention to the close correlation between the anion gap and whole blood cyanide levels in smoke inhalation-induced cyanide poisoning. They showed that anion gap and arterial pH are excellent parameters of severity, in addition to being a valuable diagnostic clue. Baud et al. (2002) found that before antidotal treatment, plasma lactate concentration correlated positively with anion gap, spontaneous respiratory rate and arterial pH.

Fall and Szerlip (2005) reported that lactic acidosis is frequently encountered in the intensive care unit. It occurs when there is an imbalance between production and the clearance of lactate. Lactic acidosis is often associated with a high anion gap and is generally defined as a lactate level more than 5 mmol/L and a serum pH less than 7.35. Lactate levels is often used as a prognostic indicator and may be predictive of a favorable outcome if it normalizes within 48 hrs. Habal and Roldan (2006) added that lactate levels greater than 8 mmol/L may serve as a cutoff level to predict survival. But specialized equipment is required for the measurement of lactate. Lactic acidosis is often associated with a high anion gap and is generally defined as a lactate level more than 10 mmol/L in patients of severe inhalation should be a suspicion of cyanide poisoning.

Dreykluft et al. (2004) reported that treatment of cyanide poisoning remains unsatisfactory because the toxicity of the antidotes may worsen the clinical picture. Nitrites result in methaemoglobin formation, which competes with cytochrome-c oxidase by virtue of its Fe^3+ atom. The reaction favours methaemoglobin because of mass action. Cyanide is converted to less toxic thiocyanate by the enzyme transsulphurase. To accelerate detoxification, thiosulfate is administered and the thiocyanate formed is excreted in the urine.

Therefore, diagnosis of cyanide toxicity may be confirmed by a variety of laboratory procedures, but accurate assay is essential for proper conclusions from analysis of animal tissues several hours after death or from human samples as fast as possible keeping the bodies and samples at 4°C until examination. The immediate laboratory confirmation in cyanide poisoning is rarely possible. The gas chromatographic procedure is not quick to perform and is, therefore, not suited to emergency situations. Drager test-tube method is a simple and fast semiquantitative analysis of cyanide in blood which can be used. The results obtained is suitable to determine the need for treatment. But specialized equipment is required for the analysis of old and grossly contaminated blood or in the analysis of tissues when blood is not available, the chromatographic procedure has to be done. However, it is good laboratory practice to use two independent methods. On the other hand, cyanide exposure can be suggested by unexplained metabolic acidosis. The serial blood lactate measurements are useful in predicting survival in fire victims with severe smoke inhalation.

REFERENCES


Baud, F.J.; S.W. Borron; B. Megarbane et al. (2002): Value of lactic acidosis in the assessment of the severity of acute cyanide poisoning, Critical Care Medicine, 30(9): 2044-2050.


