Estimating the time between drinking and death from tissue distribution patterns of ethanol.

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Abstract

To establish a method for estimating the time between the last consumption of alcohol and death, we examined the ethanol levels in body fluids and tissues of rats that had been orally administered 1 g/kg ethanol. We observed the following relationships between ethanol levels in the cardiac blood (blood in the heart itself), vitreous humor, and urine: cardiac blood > vitreous humor > urine at 10 min (early absorption stage); vitreous humor > cardiac blood > urine from 20 to 50 min (late absorption stage); vitreous humor > urine > cardiac blood at 60 to 120 min (distribution stage); and urine > vitreous humor > cardiac blood at 180 min (excretion stage). It was also observed that, in cases of death immediately following drinking, ethanol levels in the stomach contents are very high, and the following ratios of ethanol levels were observed: skeletal muscle to cardiac blood–less than 1; liver to cardiac blood–around 1. buccal mucosa to cardiac blood–greater than 1. These ratios at equilibrium after drinking were around 1, lower than 1 and around 1, respectively. We also measured alcohol levels in the cardiac blood, urine, vitreous humor and stomach contents of nine cadavers who had consumed alcohol prior to death. The relationships between the time since last consumption of alcohol and relative ethanol levels in these specimens were in good accordance with the results of the animal experiments.

KEYWORDS: toxicology, ethyl alcohol, ethanol in cadavers, tissue distribution of ethanol, time between drinking and death

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Estimating the Time Between Drinking and Death from Tissue Distribution Patterns of Ethanol

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To establish a method for estimating the time between the last consumption of alcohol and death, we examined the ethanol levels in body fluids and tissues of rats that had been orally administered 1 g/kg ethanol. We observed the following relationships between ethanol levels in the cardiac blood (blood in the heart itself), vitreous humor, and urine: cardiac blood \textgreater{} vitreous humor \textgreater{} urine at 10 min (early absorption stage); vitreous humor \textgreater{} cardiac blood \textgreater{} urine from 20 to 50 min (late absorption stage); vitreous humor \textgreater{} urine \textgreater{} cardiac blood from 60 to 120 min (distribution stage); and urine \textgreater{} vitreous humor \textgreater{} cardiac blood at 180 min (excretion stage). It was also observed that, in cases of death immediately following drinking, ethanol levels in the stomach contents are very high, and the following ratios of ethanol levels were observed: skeletal muscle to cardiac blood - less than 1; liver to cardiac blood - around 1; buccal mucosa to cardiac blood - greater than 1. These ratios at equilibrium after drinking were around 1, lower than 1 and around 1, respectively. We also measured alcohol levels in the cardiac blood, urine, vitreous humor and stomach contents of nine cadavers who had consumed alcohol prior to death. The relationships between the time since last consumption of alcohol and relative ethanol levels in these specimens were in good accordance with the results of the animal experiments.

Key words: toxicology, ethyl alcohol, ethanol in cadavers, tissue distribution of ethanol, time between drinking and death

Drinking is deeply rooted in our daily life, and many social problems and accidents are caused by alcohol. In forensic medicine, in which various kinds of unnatural deaths are investigated, postmortem determination of alcohol levels is necessary to determine whether or not the victim was under the influence of alcohol at the time of death. In some cases, the degree of intoxication at the time of death can be easily estimated by measuring the alcohol level in the cardiac blood (blood in the heart itself), but in cases where a large amount of alcohol remains in the stomach, it is possible that the alcohol level in the cardiac blood has increased by diffusion of alcohol from the stomach (1-4). If moderate or advanced decomposition has occurred, considerable amounts of ethanol may have been produced by putrefactive bacteria (5, 6), and it is important to distinguish between alcohol produced after death and alcohol consumed prior to death. In severely injured victims, alcohol production by saprogenic bacteria can occur even before death (7, 8). A useful criterion for endogenous alcohol production is the presence of n-propanol which is produced along with ethanol by putrefactive bacteria but is not normally present in the human body (9-11).

The main purpose of postmortem determination of alcohol levels is to estimate the degree of drunkenness at the time of death. The time between drinking and the death, if accurately estimated, can shed light on the circumstances and cause of death.

The time between last bout of drinking and death is generally estimated by alcohol levels in the stomach contents (12) or by relative alcohol levels in blood, urine, and vitreous humor (13-15), but there remain problems with these methods with respect to reliability. The distri-

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bution of alcohol consumed prior to death in cadavers has also been studied (16), but there have not been detailed studies on progressive changes in alcohol levels in the body fluids and tissues after alcohol intake.

In the present study, we measured changes in alcohol levels in body fluids and tissues of rats that were orally administered alcohol. Based on the results obtained, we attempted to establish a method to accurately estimate the time since last bout of drinking by relative alcohol levels in the body fluids and tissues.

Materials and Methods

**Apparatus.** A Shimadzu gas chromatograph (Shimadzu GC-5A, Kyoto, Japan) equipped with a glass column (200 cm × 0.3 cm, intradiameter) packed with 25% polyethylene glycol 1000 on shimalite 80-100 mesh and a flame ionization detector was used for ethanol analysis. The column temperature was 90°C. The temperature of the injection port and detector was 110°C. The carrier gas was nitrogen with a flow rate of 50 ml/min.

**Measurement of the ethanol concentrations in body fluids and tissues.** Ethanol concentrations were measured by head space gas chromatography with t-butanol as an internal standard (17).

**Animal experimentation.** Male Wistar rats weighing 250-350 g were used. The following experiments were performed: a) The cardiac blood, portal blood, blood in the abdominal inferior vena cava, urine, lung, spleen, kidney, liver, adipose tissue, stomach contents, femoral muscle, vitreous humor, and brain were collected in this order 10-240 min after oral administration of 1 g/kg ethanol; b) To simulate actual drinking, rats that had been orally administered 1 g/kg ethanol were readministered the same amount of ethanol 60 or 180 min after the initial administration, and the cardiac blood, portal blood, vitreous humor, liver, femoral muscle and urine were collected 10-60 min after the second ethanol administration; c) Absorbent cotton soaked with 0.5 ml of 20% ethanol was placed in the mouth for 5 min, and the buccal mucosa and cardiac blood were collected 5-50 min after its removal; and d) The same procedures as in c) were performed immediately after oral administration of 1 g/kg ethanol. The large blood vessels around the heart, portal vein and abdominal inferior vena cava were occluded with Kocher clamps to prevent mixing of blood during sampling. The esophagus and duodenum were also occluded with the clamps to prevent contamination of tissues with ethanol in the stomach.

**Forensic autopsy cases.** Ethanol levels in the cardiac blood, vitreous humor, urine, and stomach contents were measured in nine cadavers in which little decomposition had occurred and who were known to have consumed alcohol prior to death.

**Results**

**Animal experimentation.** The ethanol levels in body fluids and tissues of rats measured 10-240 min after oral administration of 1 g/kg ethanol are shown in Fig. 1. The ethanol level in the cardiac blood reached a maximum of 0.86 ± 0.07 mg/g at 30 min and then decreased to 0.05 ± 0.03 mg/g at 240 min. The ethanol level in the portal blood reached 1.07 ± 0.17 and 1.10 ± 0.15 mg/g at 10 and 20 min, respectively, and the ratio of ethanol in the portal blood to ethanol in the cardiac blood was high, at 2.08 ± 0.34 and 1.33 ± 0.06, at the same time points. The ethanol level in the portal blood then decreased to about the same level as that in the cardiac blood at 30 min (Table 1). The ethanol level in the blood in the abdominal inferior vena cava showed changes similar to that in the cardiac blood.

The ethanol level in the urine was considerably lower than that in the cardiac blood at 10 min, then increased to almost the same level as that in the cardiac blood, and then exceeded the level in the cardiac blood at 180 and 240 min (Table 1). The ethanol level in the vitreous humor remained slightly higher than that in the cardiac blood except during the very early period after ethanol administration, with its ratio to cardiac blood showed nearly constant values (1.15 ± 0.06 to 1.26 ± 0.07) between 40 and 180 min. The vitreous humor to urine ethanol ratio exceeded 2 at 10 and 20 min and then decreased to 0.89 ± 0.16 at 180 min (Table 1).

The ethanol level in the liver showed almost the same level as that in the cardiac blood at 10 min and then decreased to 1/2 or lower than 1/2 the level in the cardiac blood. The ethanol level in the femoral muscle was roughly the same as that in the cardiac blood except for the early absorption phase (Table 1). The ethanol level in the adipose tissue was low throughout the measurement period. The ethanol levels in the brain, lung, spleen, and kidney were 70-80% of the level in the cardiac blood throughout the measurement period. Disappearance of orally administered ethanol from the stomach was rapid, showing almost complete absorption within 120 min.
Fig. 1 Ethanol levels in body fluids and tissues of rats given 1 g/kg ethanol orally. Each point represents the mean of 4 or 5 animals ± SE. *: inferior vena cava blood.
Table 1  Relative ethanol levels in body fluids and tissues of rats administered 1 g/kg ethanol orally

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Portal blood/cardiac blood</th>
<th>Urine/cardiac blood</th>
<th>Vitreous humor/cardiac blood</th>
<th>Vitreous humor/urine</th>
<th>Liver/cardiac blood</th>
<th>Femoral muscle/cardiac blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.08 ± 0.34*</td>
<td>0.24 ± 0.10*</td>
<td>0.98 ± 0.09</td>
<td>6.74 ± 1.64*</td>
<td>1.17 ± 0.20</td>
<td>0.54 ± 0.06*</td>
</tr>
<tr>
<td>20</td>
<td>1.33 ± 0.06*</td>
<td>0.99 ± 0.23</td>
<td>1.82 ± 0.36**</td>
<td>2.12 ± 0.41*</td>
<td>0.56 ± 0.05*</td>
<td>0.88 ± 0.04**</td>
</tr>
<tr>
<td>30</td>
<td>1.09 ± 0.03**</td>
<td>0.98 ± 0.14</td>
<td>1.36 ± 0.12*</td>
<td>1.66 ± 0.47</td>
<td>0.49 ± 0.06*</td>
<td>0.85 ± 0.02*</td>
</tr>
<tr>
<td>40</td>
<td>1.04 ± 0.07</td>
<td>0.80 ± 0.13</td>
<td>1.24 ± 0.12**</td>
<td>1.74 ± 0.26*</td>
<td>0.49 ± 0.03*</td>
<td>0.91 ± 0.01*</td>
</tr>
<tr>
<td>50</td>
<td>1.05 ± 0.05</td>
<td>0.88 ± 0.13</td>
<td>1.24 ± 0.09</td>
<td>1.53 ± 0.24</td>
<td>0.50 ± 0.04*</td>
<td>0.90 ± 0.03*</td>
</tr>
<tr>
<td>60</td>
<td>1.01 ± 0.02</td>
<td>1.09 ± 0.19</td>
<td>1.20 ± 0.07**</td>
<td>1.43 ± 0.51</td>
<td>0.42 ± 0.10*</td>
<td>0.93 ± 0.03**</td>
</tr>
<tr>
<td>120</td>
<td>0.85 ± 0.03*</td>
<td>1.00 ± 0.12</td>
<td>1.15 ± 0.06**</td>
<td>1.18 ± 0.13</td>
<td>0.36 ± 0.02*</td>
<td>0.90 ± 0.02*</td>
</tr>
<tr>
<td>180</td>
<td>0.91 ± 0.04**</td>
<td>1.58 ± 0.24**</td>
<td>1.26 ± 0.07**</td>
<td>0.89 ± 0.16*</td>
<td>0.26 ± 0.02*</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>240</td>
<td>0.63 ± 0.22</td>
<td>7.74 ± 4.93**</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.17 ± 0.13</td>
</tr>
</tbody>
</table>

Each value represents the mean of 4 or 5 animals ± SE. ND: Not determined.

* , **: Statistically significant when mean ethanol level in each specimen was compared with that in cardiac blood (Student’s t-test, * P < 0.05; ** P < 0.1).

*Statistically significant when mean ethanol level in vitreous humor was compared with that in urine (Student’s t-test, P < 0.05).

Table 2  Relative ethanol levels in body fluids and tissues of rats readministered the same amount of ethanol 60 min after initial oral administration of 1 g/kg ethanol

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Portal blood/cardiac blood</th>
<th>Urine/cardiac blood</th>
<th>Vitreous humor/cardiac blood</th>
<th>Vitreous humor/urine</th>
<th>Liver/cardiac blood</th>
<th>Femoral muscle/cardiac blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.01 ± 0.02</td>
<td>1.09 ± 0.19</td>
<td>1.20 ± 0.07</td>
<td>1.43 ± 0.51</td>
<td>0.42 ± 0.10</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>1.48 ± 0.20**</td>
<td>0.81 ± 0.16</td>
<td>1.28 ± 0.05</td>
<td>1.94 ± 0.47</td>
<td>0.78 ± 0.10*</td>
<td>0.89 ± 0.01*</td>
</tr>
<tr>
<td>20</td>
<td>1.18 ± 0.05*</td>
<td>1.15 ± 0.09</td>
<td>1.17 ± 0.03</td>
<td>1.04 ± 0.07</td>
<td>0.72 ± 0.03*</td>
<td>0.87 ± 0.02*</td>
</tr>
<tr>
<td>30</td>
<td>0.98 ± 0.07</td>
<td>1.01 ± 0.11</td>
<td>1.28 ± 0.10</td>
<td>1.31 ± 0.14</td>
<td>0.59 ± 0.04*</td>
<td>0.87 ± 0.02*</td>
</tr>
<tr>
<td>60</td>
<td>1.04 ± 0.03</td>
<td>0.92 ± 0.18</td>
<td>1.34 ± 0.16</td>
<td>1.74 ± 0.38</td>
<td>0.65 ± 0.02*</td>
<td>0.91 ± 0.05</td>
</tr>
</tbody>
</table>

Each value represents the mean of 4 or 5 animals ± SE.

* , **: Statistically significant compared with the value at the time of ethanol readministration (Student’s t-test, * P < 0.05; ** P < 0.1).

Table 3  Relative ethanol levels in body fluids and tissues of rats readministered the same amount of ethanol 180 min after initial oral administration of 1 g/kg ethanol

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Portal blood/cardiac blood</th>
<th>Urine/cardiac blood</th>
<th>Vitreous humor/cardiac blood</th>
<th>Vitreous humor/urine</th>
<th>Liver/cardiac blood</th>
<th>Femoral muscle/cardiac blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.91 ± 0.04</td>
<td>1.58 ± 0.24</td>
<td>1.26 ± 0.07</td>
<td>0.89 ± 0.16</td>
<td>0.26 ± 0.02</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>1.39 ± 0.08*</td>
<td>0.45 ± 0.09*</td>
<td>1.59 ± 0.23</td>
<td>3.82 ± 0.57*</td>
<td>1.06 ± 0.15*</td>
<td>0.78 ± 0.07**</td>
</tr>
<tr>
<td>20</td>
<td>1.08 ± 0.03*</td>
<td>0.62 ± 0.16</td>
<td>1.39 ± 0.18</td>
<td>2.89 ± 0.69*</td>
<td>0.68 ± 0.04*</td>
<td>0.88 ± 0.04**</td>
</tr>
<tr>
<td>30</td>
<td>1.03 ± 0.06</td>
<td>0.80 ± 0.17*</td>
<td>1.19 ± 0.05</td>
<td>1.36 ± 0.63</td>
<td>0.61 ± 0.06*</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td>60</td>
<td>1.03 ± 0.04*</td>
<td>0.92 ± 0.18*</td>
<td>1.22 ± 0.07</td>
<td>1.87 ± 0.68</td>
<td>0.54 ± 0.03*</td>
<td>0.98 ± 0.06</td>
</tr>
</tbody>
</table>

Each value represents the mean of 4 or 5 animals ± SE.

* , **: Statistically significant compared with the value at the time of ethanol readministration (Student’s t-test, * P < 0.05; ** P < 0.1).

Ratios of the ethanol levels in the portal blood, urine, vitreous humor, liver and femoral muscle of rats readministered with 1 g/kg ethanol 60 or 180 min after initial administration of the same amount of ethanol to those in the cardiac blood are shown in Tables 2 and 3. In our experiment of ethanol readministration after 60 min, the
ethanol ratios of portal blood and liver to cardiac blood slightly increased 10 and 20 min after readministration, but those in the other samples were little changed. In ethanol readministration after 180 min, the ethanol ratio of portal blood to cardiac blood only slightly increased 10 and 20 min after readministration, whereas the ethanol ratio of liver to cardiac blood showed a significant increase. On the other hand, the urine to cardiac blood ethanol ratio decreased from 1.58 ± 0.24 at the time of readministration to 1/2 to 1/3 that value 10 and 20 min after readministration. The vitreous humor to cardiac blood ethanol ratio showed no significant change upon ethanol readministration, while a vitreous humor to urine ethanol ratio of more than 2 was observed 10 and 20 min after readministration. There was no notable change in the femoral muscle to cardiac blood ethanol ratio.

Figure 2 shows the ethanol levels in the buccal mucosa and cardiac blood in rats in which a piece of absorbent cotton soaked with 20% alcohol was placed in the mouth for 5 min with or without prior oral administration of 1 g/kg ethanol. In rats that had been orally administered ethanol, the ethanol levels in the cardiac blood remained constant throughout the measurement period at around 1 mg/g. On the other hand, the ethanol levels in the buccal mucosa were considerably higher than those in the cardiac blood within 20 min after removal of the absorbent cotton and then decreased to approximately the same level as that in the cardiac blood. In rats without prior ethanol administration, the ethanol level in the buccal mucosa was high, reaching 4.55 ± 1.09 mg/g 5 min after removal of the absorbent cotton and still showing 1.50 ± 0.66 mg/g at 10 min, but no ethanol was detected at 40 min. There was no increase in the ethanol level in the cardiac blood.

Forensic autopsy cases. Records from the time of drinking to death were available in cases 1–4 (Table 4). In case 1, the victim was stabbed in the chest after a long bout of drinking and died within 10–20 min. The ethanol level in the urine was slightly lower than the levels in the cardiac blood and vitreous humor. The ethanol level in the vitreous humor was slightly higher than that in the cardiac blood, in accordance with the results of the animal experiments in which the former quickly exceeded the latter after ethanol administration. The ethanol level in the stomach contents was high (18.6 mg/g).

In case 2, the victim died in a car accident approximately 1 h after drinking. The decedent took alcohol with a meal 3–4 h before death and started to drink lightly again from 1.5 h prior to death. The ethanol level in the stomach contents was high (13.4 mg/g). The ethanol levels in the other samples were in the order of urine > vitreous humor > cardiac blood, which correspond to levels expected 3–4 h after drinking.

In cases 3 and 4, the victims died 3–4 h after drinking. The ethanol levels were in the order of urine > vitreous humor > cardiac blood, with low levels of ethanol in the stomach. These indicate deaths in the excretion phase. The circumstances of drinking were unknown in cases 5–9.

In case 5, the ethanol level in the stomach contents was relatively high and the ethanol levels in the other samples were in the order of vitreous humor > urine >
Table 4  Ethanol levels in cardiac blood, urine, vitreous humor and stomach contents in 9 autopsy cases

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (Sex)</th>
<th>Time after death</th>
<th>Cause of death</th>
<th>Postdrinking interval</th>
<th>Ethanol concentration (mg/g)</th>
<th>Weight of stomach contents (g)</th>
<th>Volume of urine (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cardiac blood</td>
<td>Urine</td>
<td>Vitreous humor</td>
</tr>
<tr>
<td>1</td>
<td>64</td>
<td>12 ~ 18 h</td>
<td>Bleeding</td>
<td>10 ~ 20 min</td>
<td>2.56</td>
<td>2.52</td>
<td>2.63</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>14.5 h</td>
<td>Brain contusion</td>
<td>1 h</td>
<td>0.82</td>
<td>1.76</td>
<td>1.60</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>43 h</td>
<td>Traumatic shock</td>
<td>3 ~ 4 h</td>
<td>1.23</td>
<td>2.56</td>
<td>2.05</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>8 h</td>
<td>CO poisoning</td>
<td>3 ~ 4 h</td>
<td>1.43</td>
<td>2.00</td>
<td>1.81</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>24 h</td>
<td>Unknown</td>
<td>Unknown</td>
<td>2.66</td>
<td>3.40</td>
<td>3.50</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>10 h</td>
<td>Lung injuries</td>
<td>Unknown</td>
<td>1.75</td>
<td>3.49</td>
<td>2.38</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>24 h</td>
<td>Ruptured cardiac</td>
<td>Unknown</td>
<td>1.91</td>
<td>2.70</td>
<td>2.30</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>38 h</td>
<td>Brain contusion</td>
<td>Unknown</td>
<td>1.42</td>
<td>2.41</td>
<td>2.24</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>35 h</td>
<td>Bleeding</td>
<td>Unknown</td>
<td>1.11</td>
<td>1.71</td>
<td>1.40</td>
</tr>
</tbody>
</table>

BAC: Cardiac blood ethanol concentration; UAC: Urine ethanol concentration; VAC: Vitreous humor ethanol concentration.

cardiac blood, implying a death 1 ~ 2h after drinking.

In cases 6 ~ 9, the ethanol levels in the stomach contents were low and the ethanol levels in the other samples were in the order of urine > vitreous humor > cardiac blood, implying deaths more than 2h after drinking.

Discussion

Ameno (13) proposed the following standards for estimation of the time between last bout of drinking and death: urine to blood ethanol ratios less than 1 correspond to a death within 1h of last bout of drinking; between 1 and 1.3 to a death more than 1h after last bout of drinking; and over 1.3 to a death several hours after last bout of drinking. Yip and Shum (14) and Chao et al. (15) suggested that a postmortem vitreous humor to blood ethanol ratio of less than 1 indicates death in the early absorption phase whereas a ratio greater than 1 indicates a death in the late absorption to excretion phase. However, accuracy of these estimates is not always high in these studies.

In the present study, using rats administered with 1 g/kg ethanol orally, the following tendencies in the relationships between the ethanol levels in the cardiac blood, vitreous humor, and urine after oral administration of ethanol were observed: cardiac blood > vitreous humor > urine at 10 min (early absorption stage); vitreous humor > cardiac blood > urine from 20 to 50 min (late absorption stage); vitreous humor > urine > cardiac blood from 60 to 120 min (distribution stage); and urine > vitreous humor > cardiac blood at 180 min (excretion stage). Therefore, it appears that a more accurate estimate of the time since the last bout of drinking can be made based on the relationship between the ethanol levels in these three tissues.

Despite growing knowledge of the liver to blood ethanol ratios at equilibrium after drinking in human subjects and animals (18, 19), the relationship between the postdrinking interval and these ratios is still unclear. Our study revealed that the liver to cardiac blood ethanol ratio was 1.17 ± 0.20 at 10 min after ethanol administration, and then decreased to 0.26 ± 0.02-0.56 ± 0.05 at...
equilibrium. The lateral part of the right lobe of the liver is relatively unaffected by diffusion of drugs from the stomach (20), and the liver to cardiac blood ethanol ratio is considered to be a reliable parameter for estimation of the time since last bout of drinking.

Although a good correlation was observed between ethanol levels in the portal blood and in the liver, portal blood may not be the specimen of choice because it is very susceptible to postmortem diffusion of ethanol from the stomach (3). The femoral muscle to cardiac blood ratio was low (0.54 ± 0.06) at 10 min after ethanol administration, though it rose to around 1 between 20 and 240 min. These results suggest the potential usefulness of femoral muscle ethanol levels in judging whether deaths occurred in the early absorption phase. The ratios of ethanol levels in other tissues examined to ethanol levels in cardiac blood showed little change after ethanol administration, indicating that they are of little value in estimation of the time since last bout of drinking.

Bacsera et al. (12) drew attention to the usefulness of the ethanol level in stomach contents, and showed that stomach ethanol levels higher than 5 mg/g imply death immediately following drinking, i.e., death in the ethanol absorption phase, whereas ethanol levels below 5 mg/g imply death in the ethanol excretion phase. Our results substantiate their findings.

Estimation of the time between drinking and death is often complicated by prolonged drinking with some intervals. There is little information about effects of drinking patterns on tissue distribution of ethanol. To simulate actual drinking, rats that had been orally administered 1 g/kg ethanol were readministered the same amount of ethanol 60 or 180 min after the first administration. In our experiment of ethanol readministration after 60 min, the ethanol ratios of portal blood and liver to cardiac blood significantly increased 10 min after readministration, but the relative ethanol levels in the cardiac blood, urine, and vitreous humor remained almost unchanged. This is probably due to high levels of residual ethanol present in the stomach because ethanol was readministered in the late absorption to distribution phases. On the other hand, in ethanol readministration after 180 min, the ethanol levels in the body fluids and tissues as well as residual ethanol level in the stomach were already very low. Therefore, the urine to cardiac blood ethanol ratio decreased from 1.58 ± 0.24 at the time of readministration to below 1. However, the vitreous humor to cardiac blood ethanol ratio showed little change after readministra-
tion, while the vitreous humor to urine ethanol ratio increased from less than 1 at the time of readministration to over 1. The increase in the liver to cardiac blood ethanol ratio was more marked than in the readministration at 60 min. The femoral muscle to cardiac blood ethanol ratio remained unchanged upon readministration of ethanol probably because ethanol levels in the muscle and in the blood equilibrated quickly due to enhanced blood supply for the muscle caused by the readministered ethanol.

The buccal mucosa is exposed to very high levels of ethanol during drinking. Surprisingly, little is known about ethanol levels in the buccal mucosa after drinking. The results of our experiments in which a piece of absorbent cotton soaked with alcohol was placed in the mouth of rats for 5 min indicate that ethanol levels in the buccal mucosa may remain higher than those in blood for about 20 min after drinking irrespective of the presence or absence of alcohol in blood induced by prior drinking, suggesting the potential usefulness of ethanol levels in the buccal mucosa in estimation of the time since last bout of drinking.

Of the 9 forensic cases examined, the postdrinking intervals were clearly known in cases 1–4. In cases 1, 3 and 4, the relationships between the ethanol levels in the cardiac blood, vitreous humor, urine, and stomach contents were in good accordance with the results of these animal experiments. In case 2, in which only 1 h had passed since the last bout of drinking, the ethanol level in the urine was higher than that in the vitreous humor. This apparently contradicts the results of rats readministered ethanol. However, this contradiction may be explained by the following: a) The amount of ethanol taken in the second round of drinking was small, though not clearly known; and b) Its absorption had been delayed due to the presence of 350 g of half-digested food material in the stomach. Although the sample size of the present study is limited, oral administration of ethanol to rats may be useful as an experimental model of tissue distribution of ethanol in alcohol drinkers. Thus, the postdrinking intervals in cases 5–9 estimated from the results of animal experiments may be reliable.

In conclusion, we demonstrated the following: a) The time since last bout of drinking can be accurately estimated using the relationship between the ethanol levels in the blood, vitreous humor and urine; b) Re-drinking of ethanol in the absorption to distribution phases does not affect the relationship of ethanol levels between these
fluids whereas re-drinking in the ethanol excretion phase can affect this relationship depending on the amount of ethanol taken; and c) Not only ethanol levels in the stomach contents but also the ratios of the ethanol levels in the skeletal muscle, liver, and buccal mucosa to that in the blood are useful in judgment as to whether death immediately followed drinking.

References


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