Toxicology and fly larvae on a putrefied cadaver

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Toxicological analyses on a putrefied cadaver are sometimes difficult to achieve, due to the absence of blood and/or urine. In this study, morphine and phenobarbital were simultaneously identified and assayed in several tissues of a putrefied cadaver and in the fly larvae of Calliphoridae found on the corpse.

Key Words: Fly larvae; Putrefaction; Toxicology; Autopsy.

Revision received 4 June 1990; accepted 20 July 1990

Introduction
Fly larvae have been used in forensic medicine for many years in evaluating the time and geographical location of death. The taxonomy of larvae, their growth rate and their metamorphic development are typical parameters used to determine how long ago the death occurred.

With a putrefied cadaver, it is not always possible to obtain blood and urine samples at autopsy, a problem that has to be taken into account when drug poisoning is suspected. In 1980, Beyer et al. assayed phenobarbital in Calliphoridae larvae in a case of a fatal overdose [1]. In two previous studies we simultaneously identified five drugs (triazolam, oxazepam, phenobarbital, clomipramine and alimemazine) in human organs and in Calliphoridae larvae [2], and two drugs (bromazepam and levomepromazine) in human organs and Piophilidae [3]. The present observation develops our earlier conclusions.

Case history
On 13 July 1989, the cadaver of a man known to be a chronic heroin abuser, was found lying in his home. He had been dead since 4 July. At autopsy, we identified a 31-year-old white male. The entire corpse which was putrefied was covered with hundreds of identical larvae, recognised to be the larvae of flies belonging to the Calliphoridae family. The X-ray did not show any bullets.

Post-mortem specimens taken included heart, liver, whole blood, kidney and brain. Samples of living fly larvae were removed from different places.
on the corpse and pooled. No urine was taken, since the bladder was totally empty.

**Method**

Fifty live larvae, 12 to 17 mm long, weighing an average of 74.8 mg each, were copiously washed with deionized water, dried and stored at 4°C. Before the toxicological analyses, they were again washed and dried with filter paper and homogenized in a 0.9% saline solution using a Potter Elvehjem homogenizer. Human tissues were homogenized in the same manner. Before analysis, no other treatment such as digestion was necessary. Human and larvae liquid homogenates were pipetted in Pyrex centrifuge tubes and analyzed as fluids.

An initial screening, performed on the whole blood by fluorescence polarization immunoassay (Abbott ADx®) indicated the presence of opiates and barbiturates. No amphetamines, cocaine, benzodiazepines, antidepressants or ethanol were detected. Phenobarbital and morphine were identified by liquid chromatography and gas chromatography respectively, using two procedures previously described [4, 5]. In order to avoid possible contamination, the larval extracts were injected twice, at the beginning and at the end of the automatic session. In no case did the results differ significantly from one injection to the other.

The chromatographic results of the free-morphine and the phenobarbital assays are presented in Table 1; both drugs were found in the larvae.

**TABLE 1 Concentrations of morphine and phenobarbital in the autopsy samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Free morphine (µg/l or µg/kg)</th>
<th>Phenobarbital (mg/l or mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>380</td>
<td>5·10</td>
</tr>
<tr>
<td>Liver</td>
<td>523</td>
<td>1·61</td>
</tr>
<tr>
<td>Heart</td>
<td>297</td>
<td>0·94</td>
</tr>
<tr>
<td>Kidney</td>
<td>203</td>
<td>1·48</td>
</tr>
<tr>
<td>Brain</td>
<td>52</td>
<td>0·25</td>
</tr>
<tr>
<td>Larvae</td>
<td>182</td>
<td>0·50</td>
</tr>
</tbody>
</table>

The larvae were also tested for morphine and phenobarbital by fluorescence polarization immunoassay (FPIA) on the Abbott ADx using the reagents supplied by the manufacturer (Urine Opiates kit and Serum Phenobarbital kit). The only pre-treatment of the larvae was homogenization, centrifugation and dilution with saline. The results obtained with chromatography and FPIA are compared in Table 2.
TABLE 2 Comparison between chromatography and FPIA in assaying morphine and phenobarbital in larvae samples

<table>
<thead>
<tr>
<th>Technique</th>
<th>Morphine (µg/kg)</th>
<th>Phenobarbital (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatography</td>
<td>182</td>
<td>0.50</td>
</tr>
<tr>
<td>FPIA</td>
<td>165</td>
<td>0.56</td>
</tr>
</tbody>
</table>

FIGURE 1 Gas chromatograms obtained from a larvae extract
The good correlation observed between the two techniques is of particular importance when no human fluid is available after the autopsy. Conventional chromatographic analyses are time-consuming and require several extraction steps. By FPIA, qualitative estimation of drugs is obtained within 15 min. Nevertheless, in order to eliminate the false FPIA response, gas–liquid chromatography is useful for confirmation and quantification. Chromatograms obtained after larvae extraction have presented less endogenous peaks than those obtained with the human specimens, permitting good interpretation (Figure 1).

**Conclusion**

This study has indicated that even with a putrefactive cadaver, toxicological investigations are possible, given the presence of fly larvae. These analyses can be performed on living materials, which is always more suitable for toxicological screening. At autopsy, larvae sampling is easy, and their subsequent preparation and extraction are the same as those from any human tissue.

**References**


