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# Determination of Drug Levels in Larvae of *Lucilia sericata* (Diptera: Calliphoridae) Reared on Rabbit Carcasses Containing Morphine

**REFERENCE:** Hédouin V, Bourel B, Martin-Bouyer L, Bécart A, Tournel G, Deveaux M, Gosset D. Determination of drug levels in larvae of *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. J Forensic Sci 1999;44(2): 351–353.

**ABSTRACT:** This study concerns the determinations of morphine concentrations in fly larvae reared on rabbits administered different concentrations of morphine and a correlation between concentrations of the drug in larvae and tissues. Three rabbits (R1, R2 and R3) were given dosages of 12.5, 25.0 and 50.0 mg/h of morphine over a 3 h period via continuous ear artery perfusion. These dosages and time of perfusion were calculated to create tissue concentrations of morphine similar to those encountered in human deaths due to overdose. Morphine blood level plateau was attained after 1 h of perfusion. A fourth rabbit was used as a control. To evaluate drug concentrations, tissues were sampled using a coelioscopic technique. Approximately 400 eggs of *Lucilia sericata,* all of the same age category, were placed in eyes, nostrils and mouth of each rabbit carcass. Larvae and puparia were regularly collected from each rabbit for toxicological analysis. The concentrations of the drug in the tissues sampled were determined to be similar to those normally encountered in human overdoses and were correlated with the dosage of morphine that had been administered. Morphine was detected in all larvae and pupae fed on tissues from carcasses administered morphine, except for puparia from the colony fed on the R1 animal which received 12.5 mg/h dosage of morphine. All samples from the control rabbit were negative for morphine. Concentrations of morphine in larvae reared on rabbit carcasses containing morphine were 30 to 100 times lower than the concentrations found in the tissues. A correlation between the tissue concentrations and larval concentrations was found in only 3rd instar larvae (80 to 140 h following hatching). No correlations were found between administered dosages, tissue concentrations and younger larvae, prepuparial larvae or puparia.

**KEYWORDS:** forensic science, morphine, heroin, entomotoxicology, rabbits, forensic entomology

In cases of advanced decomposition when no tissues or body fluids are available, insects can serve as an alternative medium for toxicological analyses (1) and several cases and studies using insect larvae for toxicological analyses have already been published (2,3). In these instances, the analyses of the insect larvae have provided a qualitative assessment of the presence of a drug in the corpse. In a recent review, Goff and Lord (1) discuss the advantages and difficulties of this new area of forensic investigation. Although in an experimental study Introna et al. (4) described a correlation between the concentration of a drug in a substrate and the concentration in larvae feeding on that substrate, most workers have not observed any correlation between drug concentrations in larvae and the solid human tissues on which these larvae were feeding. Others have seen significant correlations between concentrations in liver tissue and those in the maggots (1,5–7). It must be kept in mind that the metabolism of the larvae themselves most probably plays a determinant role in the level of the drug (8–10).

In the present study, using a controlled experimental model, we investigated the concentrations of morphine in each developmental stage of the fly *Lucilia sericata* (Diptera: Calliphoridae) reared on rabbits administered different dosages of the drug. At present, the metabolism of drugs by insect larvae is poorly known and appears to vary according to the stage of development of the larvae. Additionally, we have attempted to detect a correlation between the morphine concentrations of the tissues used as a food source and the concentrations in the insects fed on those tissues.

## Materials and Methods

Three domestic rabbits (R1 = 4.45 kg, R2 = 4.5 kg; and R3 = 4.55 kg total weight) were administered dosages of 12.5, 25.0 and 50 mg, respectively, of morphine hydrochloride via ear artery perfusion over 3 h (10.1, 20.2, 40.4 mg free base morphine equivalent). Morphine hydrochloride was diluted in 150 mL isotonic saline solution for administration. These dosages and rate of perfusion were calculated to obtain morphine tissue concentrations similar to those encountered in cases of fatal human overdoses. These parameters were calculated from pharmcokinetics data from an earlier study (11). During the perfusion, a level plateau of morphine concentration was obtained after 100 min. This level was consistent with the dosage administered and remained constant for the duration of the perfusion. A fourth rabbit (R0 = 4.32 kg total weight) was used as a control and received only 150 mL of isotonic saline via ear artery perfusion.

Following the perfusion, the rabbits were sacrificed in a carbon dioxide chamber. Samples of organs and tissues (liver, kidney, spleen, fat, heart muscle, skin and pancreas) were taken by coelioscopy and analyzed to determine morphine concentrations. In order to perform the coelioscopy, skin and peritoneum were incised vertically following a 3 cm line in the sus-pubic area. Abdominal skin was pulled up using a string to create a pneumo-peritoneum and a 32 mm polyvinyl chloride (PVC) tube was introduced through the incision. The peritoneal cavity was illuminated by a cool fiberoptic light. Abdominal organs and, after an incision was made in the

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diaphragm muscle, thoracic organs were sampled through this tube. After sampling, the rabbits were sutured and blood cleaned to reconstitute the initial anatomy of the animal. Immediately following sampling, the tissues were homogenized in a Potter-Elvehjem homogenizer and then centrifuged. Two aliquots were made of the supernatant and these were stored at −20°C until analysis.

Approximately 400 eggs of *L. sericata* were placed in the eyes, nostrils and mouth of each rabbit carcass. The eggs used in this study were of a uniform age and obtained from a colony of *L. sericata* established from specimens collected from decomposing human bodies. The time at which the eggs were placed was defined as time T0. Rabbit carcasses were placed into plastic boxes covered with wire netting to prevent contamination by other insect species. The four boxes were placed in a closed room under normal daylight conditions. Temperatures during the study ranged from 20 to 22°C.

At regular intervals, random samples of ten larvae were taken from each carcass. These larvae were dried and frozen. For analysis, the larvae were homogenized in a Potter-Elvehjem homogenizer, centrifuged, and the supernatant analyzed. Morphine concentration of the samples was determined with a sensitive and specific radioimmunoassay technique (Coat-a-count Serum morphine RIA, Behring Diagnostic, Rueil, France. Detection limit: 1 ng/mL). Samples expected to have concentrations higher than the highest calibration (250 ng/mL) were diluted with human serum prior to assay. Concentrations were determined two times, following the manufacturer's instructions.

For the statistical evaluation of the results, a linear regression was used for concentrations of the drug in tissues against initial injected dosage, and concentration in larvae against concentration of the drug in muscle and fat tissues.

## Results

All blood and tissue samples from the rabbits receiving dosages of morphine hydrochloride were positive for the drug. All samples from the control rabbit were negative. For each rabbit, the concentration of morphine in tissues was consistent with the dosage administered (Table 1); however, no strict proportionality was observed. Morphine concentrations in tissues from rabbit R2 were 1 to 2.5 times higher than those for rabbit R1, and those for rabbit R3 were 1 to 2.7 times higher than for rabbit R2 (Table 1).

After T0, larvae developed as follows: 2nd instar observed at hour 69, 3rd instar at hour 86, prepuparial stage at hour 165, and puparial state at hour 236. Morphine concentrations in larvae are given in Table 2. Overall these concentrations are 45 to 100 times less than those seen in the tissues. Regression analysis shows the concentrations in larvae analyzed from hours 116 and 140 correlate well with the initial quantity of morphine administered as well as the concentrations in muscle and fat tissues used as a feeding substrate (Table 2). Tests were not done on larvae collected at hour 165 to 217, as larvae in several different stages of development were found simultaneously on the carcasses (early to late 3rd instar and prepuparial larvae).

TABLE 1—*Concentration of morphine in rabbits tissues (in ng/g).*

| Tissues | R1 | R2 | R3 | r† |
|---|---|---|---|---|
| Cardiac blood | 1812 | 3466 | 3824 | 0.90 |
| Liver | 5786 | 9214 | 12754 | 0.96 |
| Kidney | 5860 | 7210 | 13848 | 0.98 |
| Heart | 1856 | 3173 | 4017 | 0.94 |
| Spleen | . . .* | 10111 | 16615 | . . . |
| Fat | 1015 | 2115 | 5821 | 0.99 |
| Pancreas | 8109 | 8976 | 13370 | 0.87 |
| Skin | 2272 | 5740 | . . . | . . . |
| Muscle | 3113 | 4035 | 6432 | 0.96 |

* Data not available.

† r is the correlation coefficient between the injected dosage and the concentrations in the organs.

TABLE 2—*Concentration of morphine in ng/g from larval to pupal stages.*

| Time (hours) | R1 | R2 | R3 | $r_1$¶ | $r_2$** |
|---|---|---|---|---|---|
| 41 | 172.6* | 62.1* | 31.9* | 0.15 | −0.18 |
| 69 | 85.5† | 22.1† | 84.6† | 0.71 | 0.59 |
| 86 | 32.2†‡ | 37.8†‡ | 167.2†‡ | 0.89 | 0.99 |
| 91 | 72.1‡ | 47.1‡ | 129‡ | 0.94 | 0.90 |
| 116 | 64.1‡ | 79‡ | 150‡ | 0.99 | 0.96 |
| 140 | 51.5‡ | 116‡ | 201.3‡ | 0.97 | 0.97 |
| 165 | 32.5‡ | 15.6§ | 117.3‡ | . . .†† | . . . |
| 189 | 9.5§ | 0§ | 55.6§ | . . . | . . . |
| 217 | 1.2§ | 0§ | 3.7§ | . . . | . . . |
| 236–447 | 0 ‖ | 1.2‖ | 4.5‖ | 0.85 | 0.99 |

* Larvae stage 1.

† Larvae stage 2.

‡ Larvae stage 3.

§ Prepupal stage.

‖ Pupal stage.

¶ $r_1$ is the correlation coefficient between the muscle and the larval concentrations.

** $r_2$ is the correlation coefficient between the fat and the larval concentrations.

†† Not done.

## Discussion

We used the animal model detailed in Hédouin et al. (11). The aim was to use the animal model for entomological studies but not to perform pharmacokinetics analysis of postmortem morphine concentrations. We emphasize that these are single-dose studies that do not correlate with the situation that exists in most narcotic deaths when the deceased is a regular narcotic user. This model allows for close approximation of the natural conditions during colonization of a human death from drug overdose by insect larvae. Sampling of tissues using a coelioscopic method maintains the natural anatomy of the rabbit carcass and avoids an artificial migration of larvae to a large surgical wound after hatching from eggs deposited in the eyes, nostrils or mouth. This model also allows for controlled levels of drugs in blood and other tissues and approximation of visceral tissue levels of morphine similar to those encountered in fatal human overdoses (12), as confirmed by analyses. We point out that the Baselt and Cravey references are a compilation of toxicology data that include tissue concentrations that are total morphine (morphine plus morphine glucuronide). The radioimmunoassay (RIA) that we used measures only free morphine (13) but nobody knows the possible glucuronic acid conjugation of the morphine in insects.

In this study there was a good correlation between the quantity of morphine administered and the concentration in tissues, with the exception of the blood for R3. This can be explained by the fact that this rabbit was not sacrificed immediately following completion of the perfusion. This delay permitted the rabbit to eliminate some of the drug. For each organ sampled, the level of morphine

detected was within the range of normal lethal human concentrations (Table 1). The lowest level was observed in R1 and the highest in R3. The best correlations between tissue levels and dosage administered were seen in the kidneys, liver, and particularly the muscle and fat, which are normally the first tissues consumed by larvae feeding on a body.

Morphine concentrations in larvae were found to be 30 to 100 times lower than those in tissues. These results differ from those presented by Introna et al. (4) where concentrations in larvae were very similar to those in the liver tissues used. While both studies used the same analytical technique (RIA) and the same preparation of samples, there were two significant differences. In our study an entire carcass was used, while Introna et al. used only human liver tissue. There were also two different species of flies: *Calliphora vicina* for Introna et al., and *L. sericata* for the present study. Although both are in the family Calliphoridae, there may be significant differences in metabolism which may account for these differences. Other studies have found the concentrations in larvae to be significantly lower than those observed in tissues (6–8).

In this study, morphine concentrations in the larvae were significantly correlated with concentrations found in muscle and fat tissues (Table 2). These tissues are the primary food source for larvae from hours 86 to 140 following hatching. This corresponds to the late 3rd instar. Introna et al. (4) were the first to demonstrate a significant correlation between concentrations of opiates in liver tissues and larvae. In their study, larvae were fed on liver tissues from 40 human cases in which the cause of death had been determined to be opiate intoxication. Their correlation was observed during the 3rd instar larva (4). This correlation can be explained by the fact that the late 3rd instar is the period of rapid feeding by the larvae prior to entering the nonfeeding prepuparial stage. During this period, the rate of absorption of the drug by the maggot equals the rate of elimination. This is not the case in either earlier or later stages of maggot development. Following the cessation of feeding during the prepuparial stage, the concentration of the drug in the larva decreases until the puparial stage is reached. This situation has been observed in other studies involving different drugs and other species of flies. For example, Sadler et al. (14) demonstrated that larvae of *Calliphora vicina* fed continuously on amitriptyline laden muscle showed an increase in drug concentration, peaking at day 8, and then diminishing until day 16, at which time none could be detected. The analyses performed by Sadler et al. were done using high-performance liquid chromatography (HPLC). By contrast, Goff et al. (15), using a more rigorous extraction technique and analysis by gas chromatography/mass spectrometry (GC-MS) techniques, were able to detect 3,4-methylenedioxymethamphetamine in all immature stages of the sarcophagid fly *Parasarcophaga ruficornis* as well as empty puparial cases. In the present study, a small amount of morphine was still measurable in metamorphosing larvae.

Results of this study do not allow for extrapolation to human cases. Our results do indicate that detection of morphine in decomposing remains may be best accomplished by analyses of feeding 3rd instar larvae if acute morphine intoxication is suspected.

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