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Determination of Drug Levels in Two Species of Necrophagous Coleoptera Reared on Substrates Containing Morphine

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ABSTRACT: Two species of necrophagous Coleoptera: *Dermestes frischi* (Dermestidae) and *Thanatophilus sinuatus* (Silphidae), were reared on substrates containing different amounts of morphine. Colonies of *D. frischi* were reared on rabbit carcasses which had been given 10, 20, and 40 mg/h of morphine hydrochloride via ear artery perfusion over a 3 h period prior to death. A fourth rabbit served as a control. *T. sinuatus* was reared on minced beef spiked with morphine hydrochloride to give concentrations of 1000, 2500, 5000, and 10 000 ng/g and one control colony. These dosages were calculated to create tissue concentrations of morphine similar to those encountered in human deaths due to morphine overdose. Larvae, pupae, and adults (except for *T. sinuatus*) were analyzed for morphine content. All developmental stages of *D. frischi* were positive for morphine and concentrations correlated with cadaveric tissue concentrations during larval stages and to a lesser extent in the adult stage. For *T. sinuatus,* the best correlations were found in 2nd and 3rd instar larvae. This study demonstrates the potential for use of necrophagous Coleoptera, as well as Diptera larvae, as alternate specimens for toxicological analyses.

KEYWORDS: forensic science, forensic entomology, toxicology, Coleoptera, morphine

It has been well established through a series of experimental studies and cases (1–13) that maggots can be used as alternate specimens for toxicological analyses in cases of decomposed bodies when no other tissues are available for analyses. All of these records deal with fly species in the first wave of colonizing insects. These taxa occur on cadavers during the fresh, bloated, and decay stages of decomposition and cadaveric tissues may still be available for analysis during this period. During the late decay and dry stages of decomposition when tissues are not suitable for sampling, insects can still prove to be useful. Necrophagous arthropods found on bodies during these stages include larvae and adults of Diptera in the families Muscidae, Fanniidae, Piophilidae, and Phoridae, and Coleoptera in the families Silphidae, Histeridae, Cleridae, Dermestidae, and Tenebrionidae.

While some data are available concerning detection of drugs in empty Diptera puparial cases and cast skins or fecal material of Coleoptera (6,14–16), no studies have been conducted on drug levels in insects found on bodies during the later stages of decomposition. The present study deals with morphine concentrations in developmental stages of two species of necrophagous Coleoptera: *Dermestes frischi* Kugelmann (Dermestidae) (Fig. 1) and *Thanatophilus sinuatus* Fallen (Silphidae) (Fig. 2). The skin beetle, *D. frischi,* is found in various stages of development on a decomposing body over an extended period of time, including into the skeletal stage. By contrast, the carrion beetle, *T. sinuatus,* is found on the body during the active putrefaction stage. Both these species are abundant in Europe and frequently encountered in forensic investigations (17–19).

Materials and Methods

Colonies of *D. frischi and T. sinuatus* were established from specimens collected from rabbit carcasses during decomposition studies conducted in northern France.

For the study on *D. frischi,* three domestic rabbits, weighing from 3.47 to 4.08 kg, were administered dosages of 10, 20, and 40 mg of morphine hydrochloride per hour via ear artery perfusion for a period of three h. These dosages and rates of perfusion were calculated based on an earlier study (20) to give tissue concentrations similar to those encountered in fatal overdoses in humans. A fourth rabbit served as a control and received only an equivalent dose of isotonic solution. Following perfusion, rabbits were sacrificed in a carbon dioxide chamber. Samples of major organs and body fluids were taken to determine morphine concentrations. Rabbit carcasses were placed into plastic boxes covered with wire netting and held in a closed room at temperatures ranging from 20 to 22°C for approximately two months. At this point, the carcasses had reached a stage suitable for dermestid activity and approximately 50 *D. frischi* were placed on each carcass. Adult beetles were removed when a large number of larvae were observed on the carcasses. Ten active mature larvae, ten pupae, and ten first generation adults were then randomly taken on each rabbit, washed, dried, and frozen for later analysis.

The silphid *T. sinuatus* was reared on four 250 g portions of minced beef combined with morphine hydrochloride solutions to give concentrations of 1000, 2500, 5000, and 10 000 ng/g. A fifth portion was prepared as a control. Four males and six females were placed onto each portion to establish colonies. According to our ob-

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FIG. 1—Dermestes frischi *adult (size 8 mm).*

FIG. 2—Thanatophilis sinuatas *adult (size 12 mm).*

servations in experimental conditions, the development of *T. sinuatus* presents three active instars, a postfeeding stage which is the end of the third instar, and a pupal instar. After eggs hatched, ten specimens of first instar, second instar, third instar, postfeeding third instar, and pupae were sampled and prepared for toxicological analyses in the same manner as *D. frischi.*

For analysis, samples were homogenized in a Potter-Elvehjem homogenizer, sonicated for 30 min, centrifuged, and the supernatant analyzed for morphine content. Morphine content was determined using a sensitive and specific radioimmunoassay technique (Coat-A-Count® Serum Morphine, Dade Behring, France, detection limit: 1 ng/mL). Samples anticipated to have morphine concentrations higher than the highest calibration (250 ng/mL) were diluted with saline solution. Following manufacturer's instructions, assays of supernatants were made as duplicates.

Results

Morphine concentrations in rabbit tissues differed, but were proportional to the dosages administered (Table 1). Tissue concentrations ranged from 731 to 1802 ng/g, 791 to 2721 ng/g, and 1008 to 5922 ng/g, respectively, for rabbits administered 10, 20, and 40 mg/h of morphine hydrochloride. No morphine was detected in the control rabbit or insects reared on tissues from the control rabbit. Morphine concentrations were the highest in *D. frischi* larvae reared on carcasses administered 20 and 40 mg/h. They were low in pupae and adults, except for adults reared on the carcass receiving 40 mg/h of morphine hydrochloride. For every rabbit tissue, correlation coefficients were calculated between the four tissular concentrations from R0 to R3 and the four concentrations measured in larvae, pupae, and adults reared on R0, R1, R2, and R3 (Table 2). These ranged from 0.70 to 0.96 for larvae and 0.65 to 0.90 for adults, but only 0.08 to 0.42 for pupae.

For the Silphidae study (Table 3), drug concentrations were determined for the minced beef to verify the homogeneous distribution of the morphine for approximately the calculated concentrations. For all larval stages, concentrations of approximately 15 ng/g were found for larvae feeding on the 1000 and 2500 ng/g colonies and approximately 80 to 90 ng/g from the 5000 and 10 000 ng/g colonies. Concentrations observed in the 2nd and 3rd instars were proportional to the concentrations in the food source. This correlation was absent from the later larval instar and morphine could not be detected in the pupae. The best correlation coefficients were calculated from 2nd and 3rd instars (0.95 and 0.97, respectively).

TABLE 1—*Morphine concentrations in tissues of rabbits administered different dosages of morphine hydrochloride via ear perfusion over a 3 h period and in larvae, pupae, and adults of* Dermestes frischi *reared on these rabbits (R0 = control, R1 = 10 mg/h, R2 = 20 mg/h, R3 = 40 mg/h).*

Tissues	R0 (ng/g)	R1 (ng/g)	R2 (ng/g)	R3 (ng/g)
Blood	…	817	1289	1771
Muscle	…	1802	2721	5922
Hypoderma	…	1694	2345	3993
Fat	…	731	791	1008
Liver	…	1037	1832	3496
Larvae	…	0.3	24.8	71.3
Pupae	…	1.9	5.1	0.7
Adults	…	2.9	0.5	14.0

TABLE 2—*Correlation coefficients between rabbit tissues concentrations of morphine and concentrations in larvae, pupae, and adults of* Dermestes frischi *reared on these rabbits.*

Stage	Blood	Muscle	Hypoderma	Fat	Liver
Larvae	0.86	0.96	0.90	0.70	0.95
Pupae	0.37	0.08	0.22	0.42	0.15
Adults	0.74	0.90	0.83	0.65	0.87

Discussion

It is surprising that there are no data concerning drug concentrations in fresh necrophagous Coleoptera. The only study approaching this problem was that by Nuorteva and Nuorteva (21), concerning mercury bioaccumulation in Staphylinidae feeding on Diptera larvae reared from polluted fish. This study was basically done in an ecological rather than forensic context. Unlike many species of Diptera, Coleoptera of forensic significance develop on bodies when tissues are strongly decomposed. Such tissues are unavailable for toxicological analysis.

TABLE 3—*Concentrations of morphine measured in substrates containing theoretically 0 ng/g (control), 1000 ng/g (S1), 2500 ng/g (S2), 5000 ng/g (S3), and 10 000 ng/g (S4) of morphine hydrochloride, and in larvae and pupae of* Thanatophilus sinuatus *reared on these substrates.*

	Control (ng/g)	S1 (ng/g)	S2 (ng/g)	S3 (ng/g)	S4 (ng/g)	*r*
Substrate	…	1302.9	2640.5	4811.6	12025.2	–
1st instar	…	16.9	15.2	83.5	93.1	0.87
2nd instar	…	1.7	20.6	67.2	102.3	0.95
Active 3rd instar	…	12.5	28.8	122.7	209.1	0.97
Post-feeding 3rd instar	…	…	7.1	3.7	139.7	0.93
Pupae	…	…	…	…	…	–

NOTE: *r* is the correlation coefficient between morphine concentrations in instars and morphine concentrations measured in substrates.

Our selection of *D. frischi* and *T. sinuatus* for this study on morphine accumulation was due to the wide distribution of these two species in Europe. Dermestids have been collected from human bodies on several occasions (22–24) and silphids are also regularly collected from forensic situations or field studies (17–19,25,26). In the absence of Diptera larvae, Coleoptera can prove to be of value in drug detection.

In the present study, morphine was detected in all developmental stages of *D. frischi.* Morphine concentrations were proportional to dosages administered to rabbit carcasses and correlated with tissue concentrations during the larval stage and to a lesser extent during the adult stage. When Coleoptera larvae mature, they cease feeding and migrate away from the food source in preparation for pupation, similar to the situation observed for the prepuparial third instar of Diptera larvae. Sadler et al. (27–28) described drug elimination in Calliphoridae larvae between larval and puparial stages related to cessation of feeding. Earlier studies on *Lucilia sericata* (Diptera, Calliphoridae) (13,29), indicate that postfeeding larvae also eliminate morphine until the puparial stage is reached. A similar elimination was observed in *D. frischi* larvae, although small amounts of morphine were detected in the pupae. A significant amount was detected in the adults, as they feed on the cadaver and acquire the drug again following emergence from the pupal stage. Their feeding is less than for the active larvae and the accumulation of drugs by adults is not predictable. For *T. sinuatus,* the best results were observed in the second and active third instar larvae. The analyses of first instar larvae were less satisfactory due to problems in analysis. The small size of these larvae resulted in a significant dilution of the entire homogenization and results can be misinterpreted if they are close to the limit of detection for the assay procedure. During the most active periods of growth (second and active third instars), larvae accumulate the drug in proportion to the substrate concentration. Before pupation, larvae cease feeding and, as for *D. frischi* and Diptera species, the drug is excreted. No morphine was detected in the pupae of *T. sinuatus.*

The present study demonstrates the utility of Coleoptera as alternate samples for toxicological analyses. These taxa, along with other species of insects found during later portions of decomposition, may prove to be of major significance during the stages when the Diptera larvae have completed their development and left the remains.

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