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Effects of Morphine in Decomposing Bodies on the Development of *Lucilia sericata* (Diptera: Calliphoridae)

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ABSTRACT: This study concerns the effects of morphine in tissues on the rate of development of *Lucilia sericata* (Diptera: Calliphoridae) using those tissues as a food source. *Lucilia sericata* is a species of fly commonly found on human corpses in Europe during the early stages of decomposition and thus of forensic interest. Three rabbits were administered 12.5, 25.0 and 50.0 mg/h of morphine chlorhydrate via ear perfusion over a period of 3 h. These dosages and duration of perfusion were calculated to give tissue concentrations of morphine similar to those encountered in fatal human overdoses. A fourth rabbit was used as a control. Following administration of the drug, rabbits were sacrificed and 400 eggs of *Lucilia sericata*, all of the same age, were placed in the eyes, nostrils and mouth of each rabbit. Developing larvae were sampled daily to determine growth rate and weight. Puparia and emerging adult flies were also sampled. Data were analyzed using analysis of variance (ANOVA) and Student's T-test. Results of this study show that an underestimation of the postmortem interval of 24 h is possible if the presence of morphine in tissues is not considered. This study demonstrates again the necessity of considering the possible effects of drugs in tissues on insect growth rates when estimating the post-mortem interval using entomological techniques.

KEYWORDS: forensic science, morphine, rabbit, *Lucilia sericata*, Calliphoridae, forensic entomology, entomotoxicology, postmortem interval

Toxicology and entomology interact in several ways: first, in the chemical control of pests of agriculture and agents causing myiasis in cattle and other animals (1); second, in the effects of environmental pollution on growth and survival of beneficial insects (2); and third, in the recently emerging field of forensic entomotoxicology (3). Recent developments in forensic entomotoxicology have taken two paths. When a body is in the later stages of decomposition there are often no tissues remaining that are normally sampled for toxicological analysis. In these cases, insects may be used as alternative sources of material for analyses (4–9). The second area for consideration has been the effects of drugs and/or toxins in decomposing tissues on the rates of development of insect larvae feeding on those tissues. These effects can alter the times required for development and introduce an error into the estimation of postmortem intervals using entomological techniques

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(10). For this reason, it is essential to know the effects of the principal substances of abuse on the development rates of those species of flies used in forensic entomology as indicators of the postmortem interval.

The present study deals with the effects of morphine on the development of the calliphorid fly *Lucilia sericata* Meigen using a controlled experimental model. This species of fly is common in the temperate zone of the Northern Hemisphere and is frequently found associated with decomposing materials including human bodies (11).

Materials and Methods

Three domestic rabbits (R1 = 4.45 kg, R2 = 4.50 kg, R3 = 4.55 kg in weight) were administered 12.5 (R1), 25 (R2) and 50 mg (R3) of morphine hydrochloride/h for a period of 3 h via ear artery perfusion through a plastic catheter (0.7 mm diameter) placed into the main ear artery. The perfusion bottle was placed 1.75 m above the rabbit. Each dosage of morphine hydrochloride was diluted in 150 mL of isotonic saline. These dosages and rate of perfusion were calculated to obtain morphine tissue concentrations approximating those encountered in fatal human overdoses. Parameters were calculated from pharmacokinetics described in a previous study (12). A fourth rabbit (R0 = 4.32 kg in weight) was used as a control and received only 150 mL of isotonic saline via ear artery perfusion.

Following perfusion, the rabbits were sacrificed in a carbon dioxide chamber. Samples were taken of liver, kidney, spleen, fat tissue, heart, muscle tissue, skin, pancreas and blood by coelioscopy to determine morphine concentration. Samples were homogenized in a Potter-Elvehjem homogenizer, then immediately centrifuged. Two aliquots were made of the liquid component of each sample and these were frozen at -20°C until analysis. Morphine concentrations were determined by radioimmunoassay (RIA) techniques (Coat-a count[®] Serum morphine RIA, Behring Diagnostic, Rueil, France); the detection limit was 1 ng/mL (13). After sampling, rabbits were sutured to reconstitute their initial anatomy.

Eggs used in this study were obtained from a colony of *L. sericata* reared from specimens collected from decomposing bodies at the morgue in Lille. Approximately 400 eggs, all of the same age, of *L. sericata* were deposited in the eyes, nostrils and mouth of each rabbit. This was defined as the T0 time. Rabbit cadavers were placed in plastic boxes with wire netting to prevent contamination by other insects. These boxes were held in a closed room with temperatures ranging between 20 and 22°C for the duration of the study. The room was exposed to normal daylight.

Each day, 20 larvae were randomly sampled from each rabbit and divided into two lots: ten larvae were used to determine growth

rates based on weight increase; and ten larvae were measured to determine development based on increase in total length. The larvae used to determine weight were washed, dried and frozen prior to weighing. The larvae used to determine length were fixed in boiling water and then preserved in 70% ETOH to show the maximum length. Measurements were made of larvae using a 20 mm reticule and the mean value of each sample of ten larvae was used to establish the growth curves. Puparia, newly emerged adults, and adults (30 min after emergence from the puparium) were also sampled. Durations of the puparial stage were recorded. The ages of adult flies were distinguished by the following criteria: immediately following emergence from the puparial case the adults are swollen, white in color, and their wings are unexpanded. Within several minutes following emergence, the digestive tract is emptied, the cuticle becomes tanned and hardened, and wings are expanded and flattened. Statistical analyses were made using analysis of variance (ANOVA) and Student's t-test.

Results

Results are presented in Tables 1–3 and Figs. 1–3. Significant differences in mean larval lengths were observed for hours 41 and 69, and, later, for hours 91, 116 and 140 (ANOVA test: $F_{obs} \geq F_{0.99}$). In almost all cases, significant differences in mean larval lengths were observed between groups for hours 41 and 69, and for hours 91, 116 and 140 (Student's t-test: $t_{obs} \geq t_{0.01}$). However, no significant differences were observed between larvae from R0 and R2 at hour 69, or between larvae reared on R1 and R3 at hours 69, 116 and 140 (Student's t-test: $t_{obs} < 0.05$).

Maximum total lengths were recorded at hour 165 for larvae reared on R0, R2 and R3, and at hour 217 for larvae reared on R1. This corresponded to the end of larval growth and the beginning of the prepupal stage, marked by cessation of feeding, a decrease in body length and migration away from the food source. Prepuparia were first observed at hour 160 for colonies on R0 and R2, and at hour 175 for the other colonies. Puparia were first observed

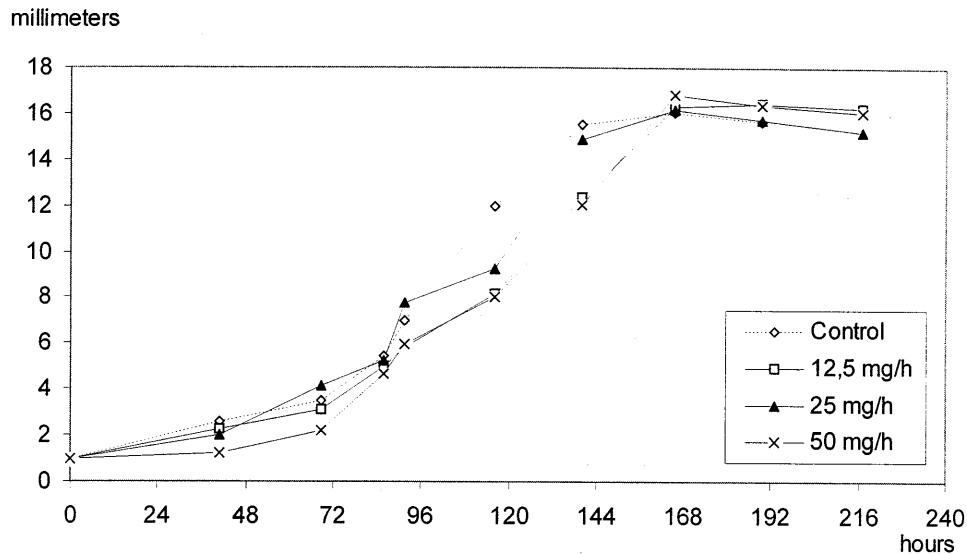


FIG. 1—Rates of development, as indicated by total body length, of *Lucilia sericata* larvae reared on rabbit carcasses receiving different dosages of morphine hydrochloride via ear perfusion over a 3 h period. R0 = control; R1 = 12.5 mg/h; R2 = 25.0 mg/h; R3 = 50.0 mg/h.

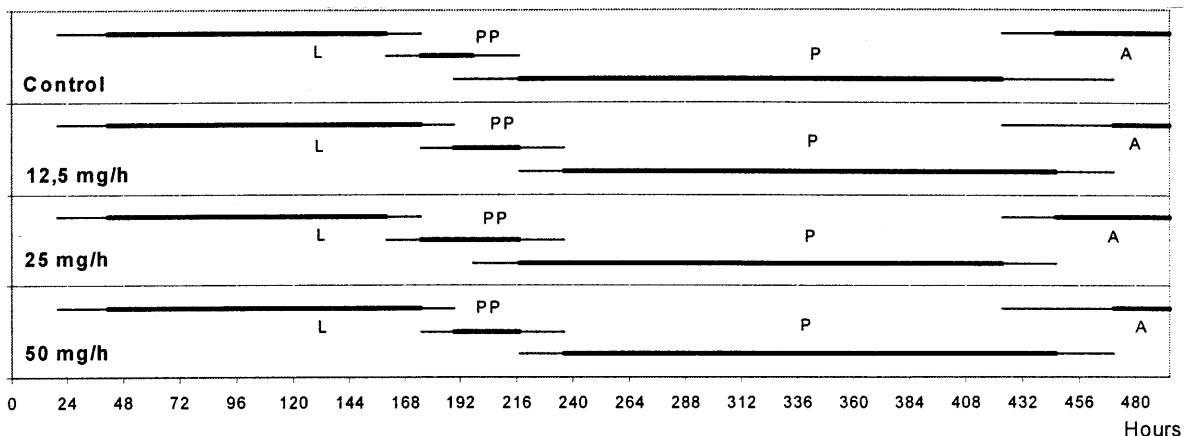


FIG. 2—Durations of larval development (L), prepupal development (PP), puparial development (P), and adult emergence (A) of *Lucilia sericata* reared on rabbit carcasses receiving different amounts of morphine hydrochloride via ear perfusion. Thickness of bands indicates relative abundance of each group over time.

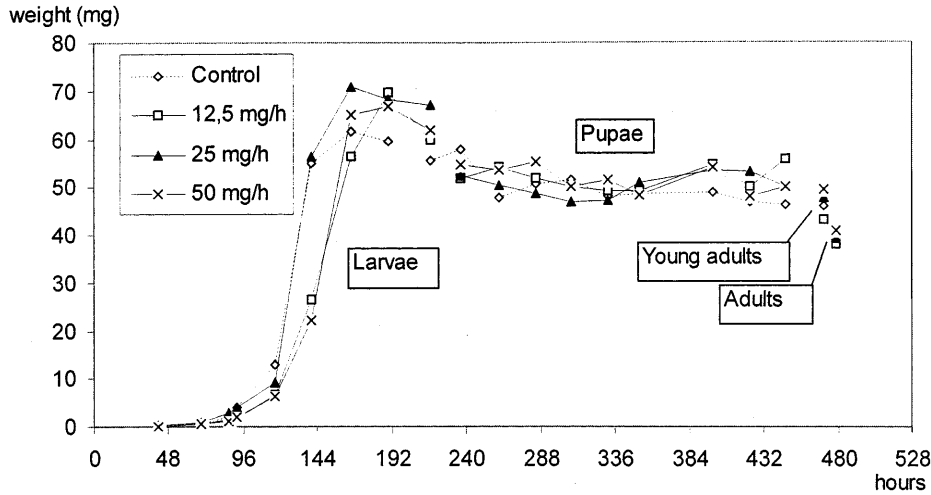


FIG. 3—Changes in weights over time of larvae, puparia, and adults of *Lucilia sericata* reared on rabbit carcasses administered different amounts of morphine hydrochloride via ear perfusion.

TABLE 1—Total body lengths and weights of *Lucilia sericata* larvae reared at ambient temperatures between 20 and 22°C on rabbit carcasses administered different dosages of morphine hydrochloride via ear perfusion. R0 = control; R1 = 12.5 mg/h; R2 = 25.0 mg/h; R3 = 50 mg/h.

Time of Sampling from T0 (hours)	R0	R1	R2	R3
41	2.6*	2.2	1.9	1.2
	0.2†	0.1	0.2	0.1
69	3.5	3.1	4.2	2.2
	0.7	0.6	0.8	0.6
86	5.4	5	5.3	4.6
	1.9	1.3	2.9	1.2
91	7	5.8	7.8	6
	4.1	1.7	4.1	2
116	12	8.2	9.3	8
	12.9	6.5	9.3	6.4
140	15.5	12.4	14.9	12
	55	26.5	56.3	22.2
165	16.1‡	16.3	16.2‡	16.8‡
	61.5‡	56.5	70.8‡	64.9
189	15.7	16.5‡	15.7	16.4
	59.5	69.7‡	68.1	66.9‡
217	...§	16.3	15.2	16.1
	...	59.9	67	62

* Length in millimeters.
 † Weight in milligrams.
 ‡ Maximum mean length or mean weight.
 § Data not available.

TABLE 2—Mean lengths and weights of puparia, emerging adults and adults of *Lucilia sericata* reared on rabbit carcasses administered different dosages of morphine hydrochloride via ear perfusion. R0 = control; R1 = 12.5 mg/h; R2 = 25.0 mg/h; R3 = 50.0 mg/h.

	R0	R1	R2	R3
Pupae	8.7*	8.6	8.5	8.6
	58†	55	54	55
Young adults	46†	43	47	49
Adults	39†	41	40	38

* Length in millimeters.
 † Weight in milligrams.

TABLE 3—Concentrations of morphine in tissues of rabbits administered different dosages of morphine hydrochloride via ear perfusion over a 3 h period. R0 = control; R1 = 12.5 mg/h; R2 = 25.0 mg/h; R3 = 50.0 mg/h.

Tissues	R0	R1	R2	R3
Cardiac blood	0	1812*	3466	3824
Liver	0	5786	9214	12754
Kidney	0	5860	7210	13848
Heart	0	1856	3173	4017
Spleen	0	...†	10111	16615
Fat	0	1015	2115	5821
Pancreas	0	8109	8976	13370
Skin	0	2272	5740	...
Muscle	0	3113	4035	6432

* Morphine concentration in ng/g.
 † Data not available.

on R0 at hour 189. Puparia were first observed in colony R2 at hour 195, and in R1 and R3 at hour 217. There were no significant differences in mean lengths of puparia among colonies (ANOVA: F obs < 0.95).

As shown in Fig. 3 and Table 1, there was a steady increase in larval weights until the prepupal stage. During this stage, weights began to decrease as the larvae stopped eating and began searching for a favorable site for pupariation. As the larvae entered the puparial stage, there was a rapid decrease in weight (Fig. 3). Mean weights further decreased during the first half of the puparial stage and then became relatively constant.

Discussion

Estimation of the postmortem interval by entomological techniques is currently widely accepted. It has also been well established that presence of drugs in decomposing tissues can alter the rate of development of insects, resulting in an incorrect estimate of the time of death. Previous works by Goff et al. (13–18) have dealt with the effects of methamphetamine, amitriptyline, 3,4-methylenedioxyamphetamine, phencyclidine, cocaine and heroin on the growth of *Boettcherisca peregrina* and *Parasarcophaga ruficornis*. These two species of Sarcophagidae are commonly found on decomposing bodies in Hawaii. Differences in

development rates for *P. ruficornis* larvae reared on liver tissues from rabbits administered different dosages of methamphetamine were sufficient to alter the postmortem interval estimates based on larval development by up to 18 h and estimates based on pupariation by up to 48 h (13). Amitriptyline produced differences in durations of larval and puparial stages among the test colonies which could result in errors of up to 77 h (15) in the estimate. By contrast, no significant differences were observed among colonies of *P. ruficornis* in the durations of the puparial stage in studies involving 3,4-methylenedioxymethamphetamine (16). Although there were differences among colonies in the duration of larval development in that study, there was no direct relation to dosages administered. While there were no differences observed in the rates of larval growth for *P. ruficornis* colonies fed on rabbit tissues containing phencyclidine, the duration of the postfeeding portion of the larval stage was shorter and the puparial stage longer (17). In the case of cocaine, the differences in growth rates for *B. peregrina* maggots fed on tissues containing cocaine and its major metabolite benzoylecognine were sufficient to result in an error of 24 h in estimation of the postmortem interval (18).

Colonies of *B. peregrina* fed on tissues containing heroin (as morphine) showed differences in larval development rates sufficient to alter the postmortem interval estimate by up to 29 h, and if based on puparial development by 18 to 38 h (19). In a previous study (unpublished data), we demonstrated that morphine in rabbit cadavers can alter the normal rate of development of *Calliphora vicina* larvae (Diptera: Calliphoridae), resulting in a 7 h underestimate of the postmortem interval (20). Both these studies were conducted under conditions far from what would be encountered in clinical situations. The rabbits received dosages that resulted in tissue concentrations far above those normally encountered in fatal heroin overdoses in humans. As heroin-related overdoses have increased in Europe over the past decade and now represent the most frequent cause of death among drug addicts, it appeared useful to continue to study the effects of morphine on insect development. This is particularly true as those bodies are rarely discovered immediately following death and questions concerning the postmortem interval are frequent.

For our study, we selected *Lucilia sericata*, which is a common necrophagous species of Diptera in Europe. Flies in the genus *Lucilia* are the familiar "greenbottles," so-called because of their brilliant metallic greenish coloration (11). Other flies commonly attracted to the body during the early stages of decomposition include *Cynomyia mortuorum*, *Lucilia caesar*, *Calliphora vicina*, *Calliphora vomitoria* and *Protophormia terraenovae* in the family Calliphoridae, and the Muscidae species *Musca domestica* and *Muscina pabulorum*.

During this study, a new experimental model was used to obtain concentrations of drugs in the rabbit tissues which were similar to those encountered in a human who had expired as the result of a drug overdose. This model allowed for the use of the entire rabbit carcass, rather than only portions of the carcass, and allowed for the complete experiment to be conducted using only a single carcass. This model allows for controlled blood and tissue levels of the drug and allows for visceral concentrations similar to those encountered in human cases, as confirmed by toxicological analyses (Table 3).

In this study, the larvae reared on the control rabbit and the rabbits receiving 12.5 and 25.0 mg/h of morphine developed at similar rates from hours 41 to 69, while those reared on the carcass receiving 50.0 mg/h of morphine developed at a slower rate (Fig. 1). From hour 91 to 165, the larvae from carcasses receiving 12.5

and 50.0 mg/h developed at the same rate and this was slower than observed from the control colony. During this same period, the larvae reared on the carcass receiving 25.0 mg/h showed an intermediate rate of growth. Thus, presence of morphine in tissues appears to retard the normal growth rate for *L. sericata* during the larval stages. This appears to be dose dependent as the larvae fed on the carcass receiving the greatest dosage were the slowest to develop. A similar relationship was seen during the active larval stages when weights were considered (Fig. 3). The decrease in mean weights of larvae during the later portion of the third instar (= prepupal stage) has been well documented for Diptera larvae. This stage is characterized by the cessation of feeding, elimination of food material from the gut, and migration by the larvae away from the food source in preparation for pupariation (22). There did not appear to be any differences in weight or length of puparia or weights of emerging and mature adults related to the presence of morphine in the tissues. These results were similar to those we obtained during earlier (unpublished) studies on effects of morphine on development of *C. vicina*. By contrast, work by Goff et al. (19) showed an increased rate of development for larvae of *Boettchersia peregrina* fed on rabbit liver tissues containing morphine, administered as heroin. This appears to indicate different responses for the sarcophagid fly, *B. peregrina*, and the calliphorid flies, *L. sericata* and *C. vicina*. Additional studies using different species of Calliphoridae and Sarcophagidae are clearly indicated as there may be different responses to the drug based on systematic differences among the Diptera.

Based on results from this study, between hours 91 and 165, estimations of larval age based on total length can be significantly in error if presence of morphine in the tissues is not considered. This error can be as great as 24 h for larvae measuring from 8 to 14 mm total length. In like manner, puparia were first observed in the control colony, while larvae in the 12.5, 25.0 and 50.0 mg/h colonies were still in the prepupal stage. Estimations of the period of insect activity based on the appearance of puparia can be significantly in error if presence of morphine is not considered. All these factors have implications in the estimations of postmortem intervals using entomological techniques.

Conclusions

The present study and earlier studies demonstrate that there are differences in rates of development of necrophagous flies when they feed on tissues containing drugs. Different species appear to have different responses to drugs and rates of development can be either increased, retarded or unchanged. In the present study, it was shown that an underestimation of the postmortem interval of up to 24 h is possible if the presence of morphine is not considered and the estimate is based on the normal development rates for *L. sericata*. This reinforces the need for further investigations of the effects of drugs and toxins in decomposing tissues on the rates and patterns of necrophagous insects using these as a food source.

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ERRATA/CORRECTIONS

We have identified a number of instances in which the authors of work published in the Journal of Forensic Sciences have miscited papers originally published in the Journal of the Forensic Science Society as having been published in the Journal of Forensic Sciences.

The known instances of this error for volume 44 of the Journal of Forensic Sciences are detailed/corrected below. We have not checked other volumes for similar errors. The Journal of Forensic Sciences regrets these errors.

Since 1995 (Volume 35), the Journal of the Forensic Science Society has been published under the title "Science and Justice."

The editors of both journals take this opportunity to remind authors of the necessity for ensuring the accuracy of the references they cite in manuscripts submitted for publication. The Instructions for Authors of both journals make it clear that accuracy of reference citation is the responsibility of authors, and good scholarship demands attention to this matter.

A. R. W. Forrest R. E. Gaensslen
Editor, Science and Justice Editor, Journal of Forensic Sciences

The journal citation in reference 7 in Foreman LA, Smith AFM, Evett IW. Bayesian validation of a quadriplex STR profiling system for identification purposes. should read: *J Forensic Sci Soc* 1992;32:5–14.

The journal citation in reference 5 in Bourel B, Hedouin V, Martin-Bouyer L, Becart A, Tournel G, Deveaux M, Gosset D. Effects of morphine in decomposing bodies on the development of *Lucila sericata* (Diptera: Calliphoridae). should read: *J Forensic Sci Soc* 1991;31:469–72.

The journal citation in reference 8 in Hedouin V, Bourel B, Martin-Bouyer L, Becart A, Tournel G, Deveaux M, Gosset D. Determination of drug levels in larvae of *Lucila sericata* (Diptera: Calliphoridae) reared on rabbit carcasses containing morphine. should read: *J Forensic Sci Soc* 1994;34:95–7.

The journal citation in reference 15 in Hedouin V, Bourel B, Martin-Bouyer L, Becart A, Tournel G, Deveaux M, Gosset D. Morphine perfused rabbits: A tool for experiments in forensic entomotoxicology. should read: *J Forensic Sci Soc* 1991;31:469–72.

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The journal citations in references 4 and 5 in Infante F, Dominguez E, Trujillo D, Luna A. Metal contamination in illicit samples of heroin. should read for 4: *J Forensic Sci Soc* 1979;19:203–9. and for 5: *J Forensic Sci Soc* 1980;20:177–81. [in reference 5 only the volume number is miscited]. And in both references, the lead author's name is "Joyce JR."

The journal citation in reference 1 in Savolainen P, Lundeberg J. Forensic evidence based on mtDNA from dog and wolf hairs. should read: *J Forensic Sci Soc* 1988;28:335–9.

The journal citation in reference 1 in Kupfer DM, Chaturvedi AK, Canfield DV, Roe BA. PCR-based identification of postmortem microbial contaminants—A preliminary study. should read: *J Forensic Sci Soc* 1968;8:73–6.

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