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Effect of Heroin in Decomposing Tissues on the Development Rate of *Boettcherisca peregrina* (Diptera, Sarcophagidae) and Implications of This Effect on Estimation of Postmortem Intervals Using Arthropod Development Patterns

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ABSTRACT: Larvae of the flesh fly *Boettcherisca peregrina* (Robineau-Desvoidy) were reared on the tissues of rabbits to study the effects of heroin on the development rates of this species. The rabbits were given 6, 12, 18, and 24 mg of heroin by cardiac puncture. From Hours 18 to 96, larvae feeding on tissues containing heroin (as morphine) developed more rapidly than those feeding on tissues from the control. The time required for pupation was significantly greater for colonies fed on tissues from heroin-dosed rabbits than for the control colony. The differences observed in the rates of development were sufficient to alter postmortem interval estimates based on larval development by up to 29 h and estimates based on pupal development by 18 to 38 h.

KEYWORDS: pathology and biology, toxicology, entomology, postmortem interval, heroin, Diptera, morphine, development rates

Over the past several years, drug-related deaths have increased in the Hawaiian Islands. These deaths are, in some instances, not discovered for a period of several days, and, because of the decomposition process, estimates of postmortem intervals are made using techniques involving arthropod development and succession patterns [*1,2*]. Concentrations of drugs or toxicants present in decomposing tissues have been determined by analyses of Diptera larvae [*3,4*]. Nuorteva and Nuorteva [*5*] have demonstrated that tissues containing mercury have an adverse effect on Diptera larvae feeding on them. With the exception of work by Goff et al. [*6*], studies have not been published concerning the effects of drugs, such as cocaine and heroin, in tissues on the development of Diptera larvae feeding on them. Because these larvae and their development patterns are used extensively in estimation of postmortem intervals in cases involving decomposing remains [*1,2*], data on the effects of these drugs on larval development become essential, partic-

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ularly in cases in the early stages of decomposition, where Diptera larvae are the predominant group present [7].

Our study concerns the effects of heroin in tissues on the development of the sarcophagid fly *Boettcherisca peregrina* (Robineau-Desvoidy). This species of fly has frequently been associated with decomposing human remains on the island of Oahu, in the Hawaiian Islands [1,8].

Materials and Methods

Four domestic rabbits (2.47 to 3.18 kg in weight) were given dosages of 6, 12, 18, and 24 mg of heroin in 5 mL of normal saline by cardiac puncture to produce different concentrations of heroin in tissues. A fifth rabbit (3.04 kg) was used as a control and received only 5 mL of normal saline by cardiac puncture. The animals were sacrificed 20 min after administration of the drug in a carbon dioxide chamber.

Immediately after death, a 2-mL blood sample was taken from each rabbit and frozen for later analysis of the heroin content. The livers and spleens were also removed from all the rabbits. The livers ranged from 74 to 104 g, with a mean of 90.8 g, and the spleens from 13 to 23 g, with a mean of 17.6 g. A sample of each liver was frozen for later analysis of the heroin content. The blood samples and liver tissues were analyzed by the Chemical Toxicology Institute, Foster City, California, using a radioimmunoassay process for heroin and its metabolites.

The flies used in this study were from a stock colony of *Boettcherisca peregrina* (Robineau-Desvoidy) established in 1984 from specimens collected during decomposition studies on the island of Oahu [7]. The livers were all exposed to this stock colony for 2 to 3 min for larviposition. The colonies thus initiated were maintained in the laboratory at 23°C in a Labline Ambi-Hi-Low environmental chamber. From this point on, the colonies will be referred to by the dosage of heroin administered (6, 12, 18, or 24 mg and the control). At 6-h intervals, the total lengths of 10 larvae from each colony were recorded to determine the growth rates. At 24-h intervals, a sample of 10 larvae was removed from each colony and frozen for later analyses of the heroin and metabolite contents. After completion of larval development, the pupae were observed at 6-h intervals, and the adult emergences were recorded. The emerging adults were maintained in separate cages and provided with a standard diet of sugar, protein hydrolysate, and water. Thirteen days after eclosion, liver was provided to each colony for larviposition. The data were analyzed by analysis of variance (ANOVA) and Waller-Duncan multiple range tests [9].

Results

The analyzed blood and liver samples showed the presence of morphine and codeine in all the rabbits that had received heroin. The sample from the control rabbit was negative for both substances. The blood sample from the rabbit dosed with 6 mg of heroin had morphine present at 436 ng/mL and codeine at 42 ng/mL. Blood from the rabbit dosed with 12 mg of heroin had morphine present at 1148 ng/mL and codeine at 144 ng/mL. The blood sample from the rabbit dosed with 18 mg of heroin had morphine present at 1451 ng/mL and codeine at 137 ng/mL. The sample from the rabbit dosed with 24 mg of heroin showed morphine at 2216 ng/mL and codeine at 228 ng/mL.

Qualitative analyses were made of samples of 10 larvae from each colony at 24 and 48-h sample periods. A cutoff value of 300 ng/mL was used to establish a positive finding. Larvae reared on tissues from rabbits dosed with 12 or 18 mg of heroin were negative for opiates at 24 h but positive at 48 h. Larvae reared on tissues from the rabbit dosed

with 24 mg of heroin were positive for opiates at both the 24 and 48-h sample periods. Both the control and the 6-mg colony larvae were negative at both sample periods.

All the tissue samples were highly attractive to adult *B. peregrina*, and larviposition began immediately upon exposure of the livers to the flies. Because of the large numbers of larvae produced, no actual counts of larvae in each sample were attempted. Previous published [*6*] and unpublished work in the Forensic Entomology Laboratory, University of Hawaii at Manoa (Honolulu, Hawaii) has indicated that exposure of a sample of liver to a stock colony of *B. peregrina* for 2 to 3 min will result in the deposition of a sufficient number of larvae for this type of study (200 to 500 larvae).

The rates of development, as indicated by the total length measurements of larvae, were not significantly different among samples of larvae from Hours 0 to 12 (Figs. 1 and 2); the mean lengths ranged from 2.7 to 3.0 mm. This period corresponded roughly to the first half of the first stadium. Beginning at Hour 18, significant differences ($P < 0.05$) were observed in the rates of development of larvae from the heroin-containing colonies when compared with the control colony. This difference continued through Hour 96, when the maximum total lengths were recorded for larvae from all the colonies (Figs. 1 and 2). Larvae from all the colonies containing heroin were significantly larger ($P < 0.05$) than larvae from the control colony, with the maximum length (17 mm, $\overline{X}$ = 16.4 mm) recorded from the 12-mg colony. The mean maximum lengths for the other colonies were the following: control, 14.2 mm; 6 mg, 15.5 mm; 18 mg, 15.3 mm; and 24 mg, 15.3 mm.

The prepupal stage, marked by a decrease in the total body length and emigration away from the food source, was first observed in the 18-mg colony at Hour 84, in the 24-mg colony at Hour 90, in the 12 and 6-mg colonies at Hour 96, and in the control colony at Hour 108. Pupation was first observed in the 24 and 18-mg colonies at Hour 126, in the 12 and 6-mg colonies at Hour 132, and in the control colony at Hour 138. The pupal weights were not significantly different between colonies, ranging from 4.5 to 6.2 mg, although the control colony contained the lighter pupae. The pupal lengths,

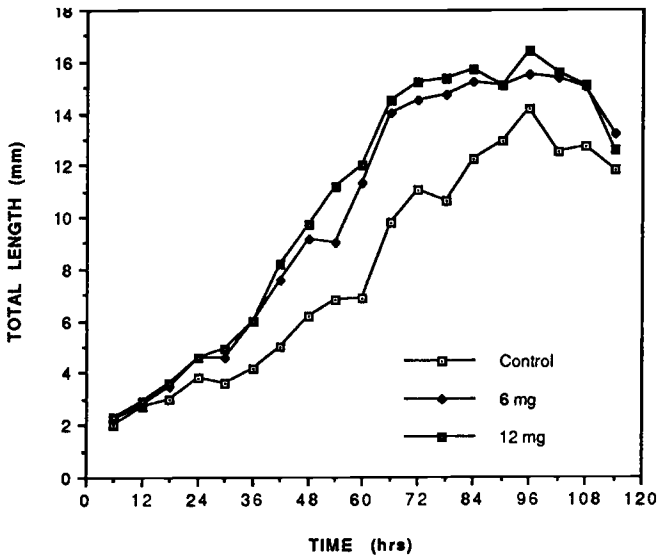

FIG. 1—*Rates of development, as indicated by total body length, of larvae of* Boettcherisca peregrina *reared on liver tissues from rabbits dosed with 6 and 12 mg of heroin by cardiac puncture.*

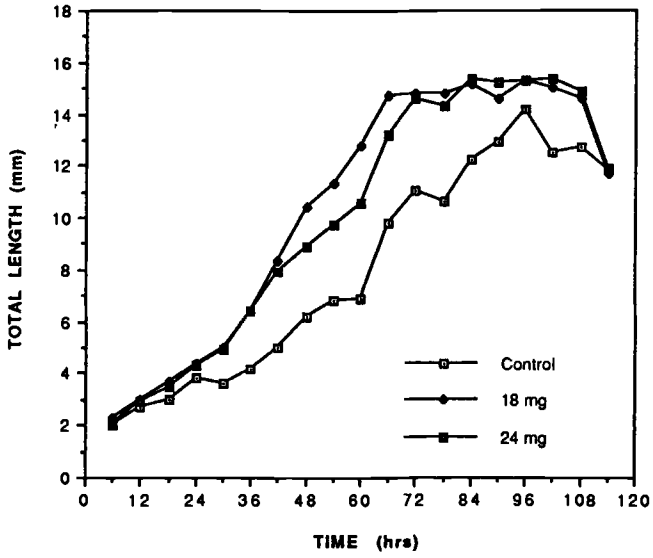

FIG. 2—*Rates of development, as indicated by total body length, of larvae of* Boettcherisca peregrina *reared on liver tissues from rabbits dosed with 18 and 24 mg of heroin by cardiac puncture.*

however, were significantly different ($P < 0.05$) between the control colony ($\overline{X}$ = 7.9 mm, 7.5 to 8.5 mm) and the colonies fed on tissues containing heroin (6 mg, $\overline{X}$ = 9.9 mm, 9 to 10 mm; 12 mg, $\overline{X}$ = 9.9 mm, 9 to 10 mm; 18 mg, $\overline{X}$ = 9.0, 8.0 to 9.5 mm; 24 mg, $\overline{X}$ = 9.6 mm, 8 to 10 mm). The total numbers of pupae, the adult emergence, the durations of the pupal stadium, and the total durations of the development period from larviposition to adult are given in Table 1. The duration of the pupal stage was significantly greater for colonies fed on tissues containing heroin than for the control colony, as was the total period required for development from larva to adult. The period required for development increased directly with the concentration of the drug in the tissues, with the exception of the 18-mg colony (Table 1). In this colony, development was more rapid than was observed in the 12-mg colony, although the rate was not significantly greater ($P > 0.05$). There were no significant differences between colonies in pupal mortality. Adults from all colonies produced viable larvae when supplied with liver 13 days after eclosion.

Discussion

The presence of heroin, as morphine, in rabbit liver tissues shortened the durations of the larval stadia of *B. peregrina* feeding on them. Unlike the situation reported by Goff et al. [6] detailing the effects of cocaine on the rate of development of *B. peregrina*, there was not a detectable difference in the rates of larval development related to different concentrations of the drug, and all 4 colonies developed at similar rates. Detectable differences between the control colony and the heroin-containing colonies in the rates of development were observed earlier (Hour 18) than was the case for larvae feeding on tissues containing cocaine. In that study [6], detectable differences first appeared at Hour 30 and corresponded roughly to the time between molt and the second instar. In the present study, significant differences were observed during the first instar. Rapid development continued until maximum size was attained and the prepupal period began. In the present study, there was a significant difference in the maximum size of larvae between

TABLE 1—*Adult emergence, pupal duration, and total development period for colonies of* Boettcherisca peregrina *reared on rabbit liver tissue containing varying amounts of heroin.*[a]

Colony	Total Pupae, *N*	Total Adults, *N*	Emergence, %	$\bar{X}$ Duration of Pupal Stage (Range), h	$\bar{X}$ Total Development Period (Range), h
Control	451	402	89	253 (246–276)*a*	397 (378–432)*a*
Dosed, mg					
6	209	198	95	271 (254–282)*b*	406 (386–450)*b*
12	150	133	88	279 (272–282)*c*	422 (404–450)*c*
18	338	328	97	273 (272–312)*b,c*	411 (404–456)*b,c*
24	310	266	89	291 (288–294)*c*	439 (408–450)*c*

[a]Figures in a column followed by the same italic letters are not significantly different ($P > 0.05$).

the control colony and the colonies feeding on tissues containing heroin. This is in contrast to the situation observed for larvae feeding on tissues containing cocaine [*6*], where there was no significant difference in maximum size between the control and test colonies. There was no significant difference between the control colony for the present study and the control colony for the cocaine studies. The flies used in both studies were from the same parent colony [*6,7*].

Pupation occurred earlier in colonies feeding on tissues from rabbits dosed with heroin than in the control colony, and pupation occurred earlier in the colonies containing the higher concentrations of heroin (as morphine). The duration of the pupal stage was significantly longer for larvae reared on tissues containing the drug than for the controls, and adult emergence was first recorded for the control colony. This was followed by the 6-mg colony, and the 24-mg colony exhibited the longest duration for the pupal stage. This is a different pattern from that exhibited by colonies reared on tissues containing cocaine or benzoylecognine, or both, where there were no detectable differences between the colonies in the duration of the pupal stage [*6*].

Our results have an important implication for the estimation of postmortem interval by entomological techniques in cases involving heroin. The levels of morphine detected in blood samples from the rabbits in this study are within the range reported by Spiehler and Brown [*10*] for heroin-related deaths and below the levels reported by Baselt [*11*]. Thus, the probability of maggots feeding on tissues from heroin related deaths ingesting quantities of morphine would appear to be high. Basic to the use of arthropod development for estimation of a postmortem interval is the relative constancy of duration of the stages of development of the species involved [*1,2*]. The species with the longest presence on the remains, as indicated by developmental stage and succession patterns, is generally the indicator of the minimum postmortem interval [*1,2*]. Goff et al. [*6*] have demonstrated that the presence of cocaine, its major metabolite benzoylecognine, or both, in decomposing tissues can accelerate the rate of larval development by up to 12 to 18 h between Hours 30 and 78 and accelerate the pupal period, correspondingly, by 12 to 18 h. In the present study, administration of heroin, in tissues as morphine, produced even more striking differences, with an acceleration of development, as indicated by the total larval length, of up to 29 h during the larval stage and pupation occurring 6 h earlier. Once pupation had occurred, the pattern reversed, and the mean durations of the pupal stage were 18 to 36 h longer than those of the control. Correspondingly, the total periods of time required for development from the larval to the adult stages were longer in colonies feeding on tissues containing heroin (as morphine). Thus, lacking data on the presence of heroin in tissues used as a food source by developing larvae, an estimate based on the normal development patterns for *B. peregrina* could be significantly different

from the actual postmortem interval. If based on larvae, the estimate would be greater than the actual interval because of the accelerated rate of development during that stage. If based on adult emergence or pupal duration, because of the retardation of development during pupation, the estimate would be shorter than the actual interval.

Although, by the limited nature of this study, these data are of a preliminary nature and for only one species of Diptera, it is reasonable to assume that similar variations also exist for other species of flies when feeding on decomposing tissues containing heroin. In like manner, similar or different effects may exist when other drugs or toxicants are present in tissues, as has been demonstrated by Goff et al. [6] for cocaine. Further investigations of the possible effects of such substances in tissues on arthropod development are clearly indicated. Until appropriate baseline data are available, care must be taken in interpretation of arthropod development and succession patterns in cases where drugs or toxicants are a factor.

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